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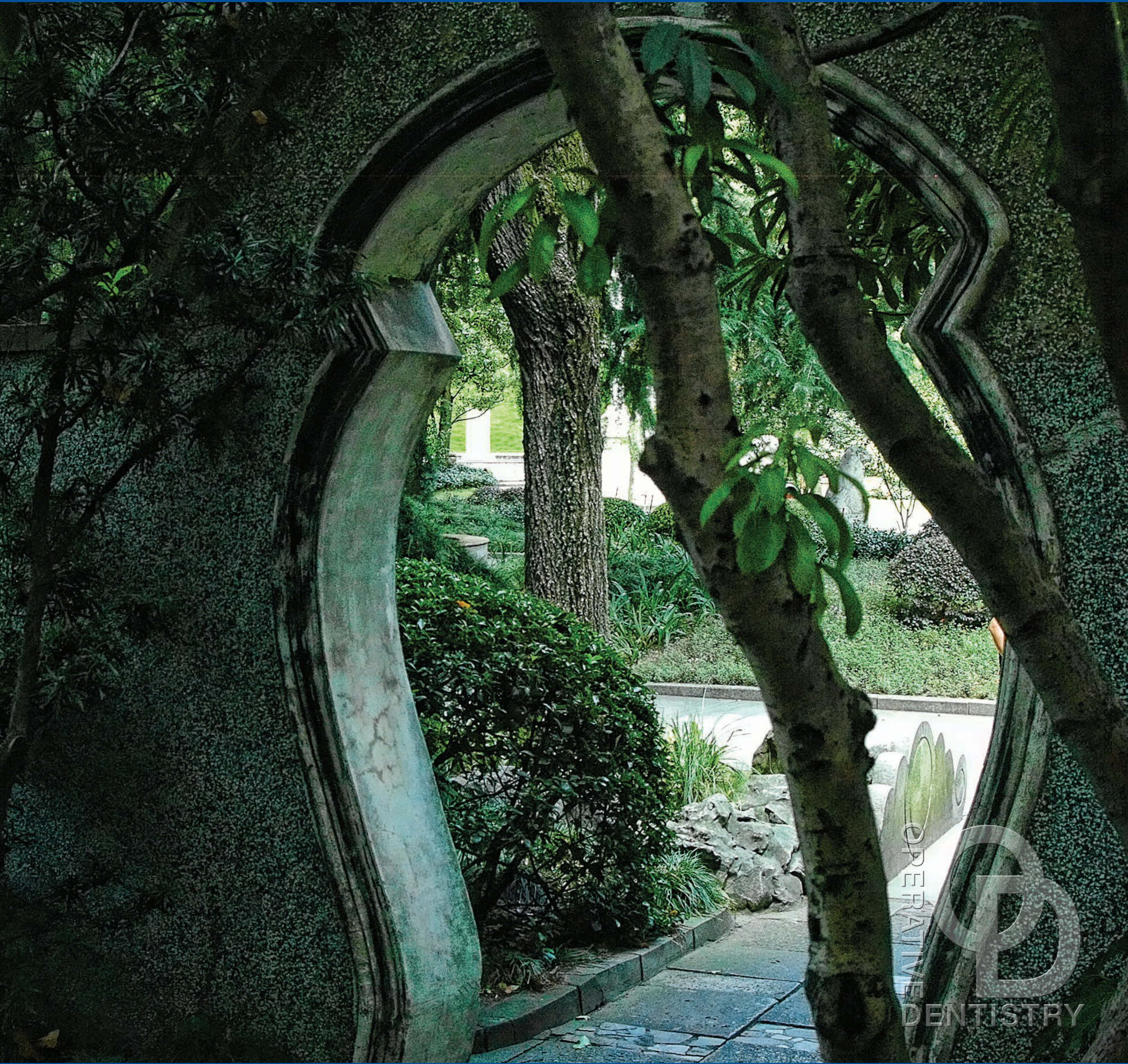

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OPERATIVE DENTISTRY

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Operative Dentistry publishes articles that advance the practice of operative dentistry. The scope of the journal includes conservation and restoration of teeth; the scientific foundation of operative dental therapy; dental materials; dental education; and the social, political, and economic aspects of dental practice. Review papers, book reviews, letters and classified ads for faculty positions are also published.

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We thank all our reviewers for their time and dedication to Operative Dentistry.

On The Cover

Doorway out of a neighborhood park in Shanghai, PRC. Photo provided by Kevin Matis of Indianapolis, IN USA. Photo taken with a Nikon D70, 28mm f/8 at 1/50 sec exposure.

We welcome the submission of pictures for consideration for use on the cover of Operative Dentistry! All photographs should be submitted via the forms at:

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A New Look

Jeffrey A. Platt, Editor

Welcome to the fortieth volume of Operative Dentistry! All who are involved with the journal are excited to bring you a new look as we continue to be among the leaders in publishing dental literature and research. As we move into 2015, you will see that several important things have happened related to your journal.

Most obvious is the change in our cover. We recognize that many of our readers are also excellent photographers and see the journal cover as an outlet for that talent. We are welcoming photograph submissions to be considered for use. Our website (https://www.jopdent.com/journal) contains the information needed to accomplish the submission. An anonymous committee has been created to vote on and select photographs that will be used on our future issues.

On our electronic outlets and social media sites, you will see our new logo. This is another way of creating a unique identity that reflects a commitment to producing a high quality competitive journal.

Only subscribers have access to the most recent three years of articles, but now anyone can access all of the journals articles that are older than three years. This is a huge accomplishment for a small journal and we should all be grateful for the staff efforts required to get it done.

The 2014 Annual Session of the Academy of Operative Dentistry included the Buonocore Lecture given by Dr. Leo Tjäderhane. This issue contains an article that includes the interesting information that was presented. You will also find ten year results for repaired amalgam restorations, several articles addressing bonding to zirconia as well as tooth structure, additional information on the impact of bleaching agents on dental structures, and an interesting look at the potential for photobiomodulation. Thank you for picking up this issue of Operative Dentistry and enjoy.

2014 George M. Hollenback Memorial Prize for Research

Dr. Edwina A.M. Kidd

Dr. Edwina Kidd is Emerita Professor of Cariology at King's College, London. She received her BDS at the Royal Dental Hospital in Lon- don in 1966, followed in 1967 and 1970 by her LDS RCS and FDS RCS degrees at the Royal College of Surgeons of England. In 1975 she did her PhD in Cariology at the University of London with Leon Silverstone, and since then her research interests have been in cariology. She held the Colgate Visiting Professorship in New Zealand in 1997. She received her DSc (Med) at the University of London in 2002. Dr. Kidd became a Professor of Cariology in 1995. Although she was a teacher of Conservative Dentistry, her general focus was on cariology and translation of new research findings into daily clinical practice.

She has published extensively in research journals and lectured worldwide. In addition to peer-review for many scientific journals, she has served as long-time member of the Editorial Board of our own journal, Operative Dentistry, and of the International Endodontic Journal, Dental Update, and the American Journal of Dentistry. Among the numerous honors bestowed for her work have been the Medical Writers Group of the Society of Authors Prize (1997), The Cartwright Prize of the Royal College of Surgeons of England (2000), the IADR Distinguished Scientist Award (2005), and the John Tomes Prize of the Royal College of Surgeons (2005). Edwina Kidd authored the handbook Essentials of Dental Caries (Oxford University Press) and co-authored the internationally acclaimed and widely-used handbook Dental Caries (Blackwell-Munksgaard) with Ole Fejerskov. As an acknowledged leader in the field, her principal aim in teaching and writing has been to base the practice of Conservative Dentistry on the science of Cariology.

Edwina Kidd qualified at the Royal Dental Hospital, London, in 1966 – almost 50 yrs ago!! After a lengthy and fulfilling career – teaching Conservative Dentistry in her beloved London, she retired in 2004 and is currently Emerita Professor at the joint London dental schools. Following are some special highlights to paint her unique persona, for those who never before had the pleasure to meet her.

As one of our English colleagues once mentioned when I made a remark about her writings: *Edwina has a way with words*. She is a gifted and prolific scientific writer. She received great accolades for her writings and essays. Her articles make an enjoyable reading, as she writes in an easy to understand manner and very clearly makes her point, connecting the science with its impact on daily practice. Even more importantly, she exemplified one of the pertinent qualities of an engaged learner and an inspiring teacher: that is: *asking the right questions*.

As no one else, Edwina was committed to create an active teaching environment and the assessment that comes with it. Not only focussing on student assessment, she created also an academic audit system to enable students to assess their teachers, with the idea that this may improve the quality of the teaching and thus student learning. No wonder, as one of the first among us, she taught integrated treatment planning teaching operative dentistry within the context of cariology so that students would apply both *non-invasive* and *operative* management appropriately.

Her first refereed publication appeared in 1974 and since then her publication flow has never ceased. Fast-forward to today, 10 yrs into retirement, Edwina is still making an impact in the field of Cariology, editing and contributing to 4 major handbooks that guide generations of students – future colleagues. Wonderful resources for the many academics among us!

As only the third women-scientist to receive this prestigious Prize (out of 40), it is especially notable that the long list of invited lectures in her CV starts with one entitled: “Women in Dentistry”. No one at that time, would have imagined that today - about 40 years later - we celebrate one of the great teacher-scientists who straddled the fields of Operative Dentistry and Cariology and had such an impact on our daily practice.

Soon after the “dental suffragettes and emancipation” starter, she focused on dental caries. Her interest and research led to the important question: *“What constitutes dental caries?”* followed by *“How ‘clean’ must a cavity be before restoration?”* and in the end: “Does it matter?” . . . and then – based on evidence – she dared to conclude “*Well, it doesn’t*!” But what does matter today is that we celebrate Dr. Kidd’s outstanding contributions to the field of operative dentistry, laid down in more than 80 publications, more than 40 review papers, and 3 books. Her writings have become classics in Operative Dentistry. Through her research, teaching and writing, Dr. Kidd is best known for her continuing quest to find the evidence in support of our daily clinical practice.

Edwina’s pursuit of excellence in Operative Dentistry has expanded the world of operative dentistry by embracing cariology. She has been, and still is, a beacon (sometimes a much appreciated feisty beacon) in the confusing world of caries removal and its management.

It is a tremendous pleasure and honor to present this award to Dr. Kidd, especially in front of this particular audience – members of the Academy of Operative Dentistry. We are elated to have Dr. Kidd with us and thank her for the numerous and truly remarkable contributions that keep our discipline grounded in science. The Academy is most pleased to recognize the exceptional work and lifetime achievements of Edwina Kidd. Her contributions have greatly advanced the field of Operative Dentistry and truly enhanced modern, quality dental practice. The Academy of Operative Dentistry is honored to announce Dr. Kidd as the most worthy recipient of the 2014 George M. Hollenback Memorial Prize.

Mathilde C Peters

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Buonocore Lecture

Dentin Bonding: Can We Make it Last?

L Tjäderhane

Clinical Relevance

Bond strength to dentin decreases with time because of the hydrolytic degradation of the hybrid layer components dentin collagen and adhesive resin. Inhibition of enzymes responsible for the collagen degradation may improve the bond strength durability.

SUMMARY

In dentin bonding, contemporary dental adhesive systems rely on formation of the hybrid layer, a biocomposite containing dentin collagen and polymerized resin adhesive. They are usually able to create at least reasonable integrity of the hybrid layer with high immediate bond strength. However, loss of dentin-bonded interface integrity and bond strength is commonly seen after aging both *in vitro* and *in vivo*. This is due to endogenous collagenolytic enzymes, matrix metalloproteinases, and cysteine cathepsins, responsible for the time-dependent loss of hybrid layer collagen. In addition, the hydrophilic nature of adhesive systems creates problems that lead to suboptimal hybrid layers. These problems include, for example, insufficient resin impregnation of dentin, phase separation, and a low rate of polymerization, all of which may reduce the longevity of the bonded interface.

Preservation of the collagen matrix integrity by inhibition of endogenous dentin proteases is key to improving dentin bonding durability. Several approaches to retain the integrity of the hybrid layer and to improve the long-term dentin bond strength have been tested. These include the use of enzyme inhibitors, either separately or as incorporated into the adhesive resins; increase of collagen resistance to enzymatic degradation; and elimination of water from the interface to slow down or eliminate hydrolytic loss of the hybrid layer components. This review looks at the principles, current status, and future of the different techniques designed to prevent the loss of hybrid layer and bond strength.

INTRODUCTION

Dentin bonding is a form of tissue engineering, in which mineral is replaced with resin monomers to form a biocomposite comprising dentin collagen and cured resin. The adhesive-dentin interface is expected to form a tight and permanent connection between dentin and composite resins.

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Dentin bonding can be accomplished with etch-and-rinse (ER) or self-etch (SE) adhesives. The common issue for both is to create a route for adhesive resin infiltration into collagenous matrix. In ER bonding systems this pathway is created with acid, which dissolves the minerals to the depth of 5-10 μm and leaves the highly porous dentin collagen network suspended in water. Then the collagen network is infiltrated with resin monomers.[1] SE systems contain acidic resin monomers that simultaneously etch and prime the dental substrate.[2] Adhesives contain solvents (water, ethanol, or acetone) to dissolve the monomers, maintain the expanded state of the collagen network, and allow the monomers to fill the spaces within and around the collagen fibrils. Chemical polymerization of these monomers, activated by the curing light, results in a polymer-collagen biocomposite, commonly called the hybrid layer.[1,2]

The hybrid layer is a highly organic interface that is relatively hydrophobic, acid resistant, and tough. However, regardless of the system or material used, the creation of the hybrid layer is not perfect.[1,2] The morphological, physiological, and pathological heterogeneity of dentin[3,4] (Figure 1); limited time available for the procedure; water required to keep the collagen network open for resin penetration[5]; and limited degree of conversion (rate of polymerization) are the major obstacles to repeatedly achieving uniform dentin bonds. Additionally, hydrolytic (water-related) degradation of both components of the hybrid layer, the collagen matrix and the adhesive resin, seriously compromises the long-term integrity of the adhesive interface and the durability of the bond strength.[1,6] Biodegradation of resin-dentin interfaces also increases bacterial microleakage,[7] leading to undetected secondary caries.

Contemporary Adhesive Systems

Traditional three-step ER adhesives use primers containing hydrophilic monomers and solvents. They aim to displace water and prepare the collagen scaffold for the infiltration of the solvent-free, hydrophobic bonding resin.[1,8,9] Simplified two-step ER systems combine the hydrophilic primer and the hydrophobic resin into one solution. In addition, SE adhesives are subdivided into two-step and one-step categories.[2] Although the simplified two-step ER and one-step SE adhesives may be more user-friendly, it is widely acknowledged that three-step ER and two-step SE systems produce more favorable results.[1,2,8-11]

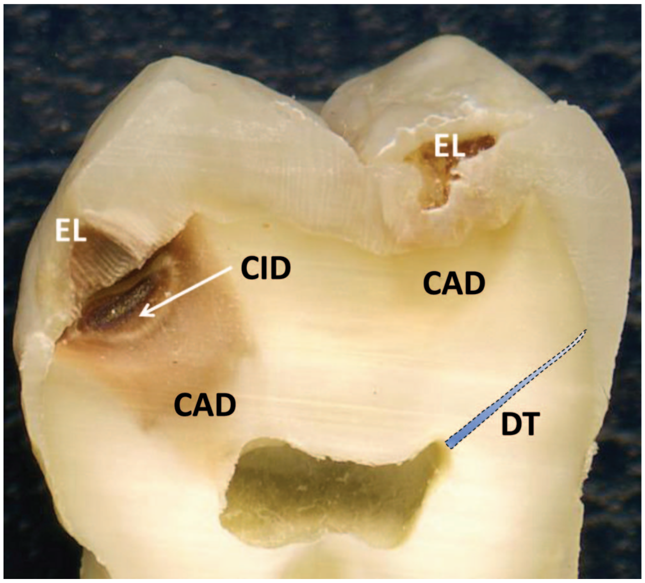

Figure 1. *The common phenomena in dentin, all of which affect the dentin as the substrate for bonding. Mineral content and the structure and composition of dentin in caries-affected dentin (CAD) and especially in caries-infected dentin (CID) is different from intact dentin, to various degrees, depending on the location. Dentinal tubules (DTs) are wide at the dentin-pulp border and gradually narrow toward the dentin-enamel junction, with the increasing amounts of highly mineralized peritubular dentin. As the tubule diameter decreases, the intrinsic wetness and dentinal fluid flow into the bonded surface decrease. EL, enamel lesion; CID, caries-infected dentin; CAD, caries-affected dentin; DT, dentinal tubules.*

DENTIN AS A SUBSTRATE FOR BONDING

To understand the problems involved in dentin bonding, we need to provide a brief overview of the dentin structure and composition to understand the complexity of the tissue in relation to the adhesion and adhesive performance. Approximately 50 vol% of dentin is mineral, the rest being type I collagen and noncollagenous proteins (30 vol%) and water (approximately 20 vol%).[3] Intertubular dentin contains well-organized mineralized collagenous organic matrix. Dentinal tubules have an inverted-cone shape, narrowing from the dentin-pulp border toward the dentin-enamel junction (DEJ) (Figure 1). Each tubule contains highly mineralized peritubular dentin, the amount of which increases toward the DEJ. Therefore, in cavities, the relative tubular and intertubular dentin areas vary depending on the depth and location of the cavity. Since dentin permeability depends on the size and patency of dentinal tubules, regional variations in dentin permeability and intrinsic wetness (due to dentinal fluid) depend strongly on the location of the cavity.[12] These variations in dentin structure and permeability directly affect bonding.[1,9,10,13] Higher surface moisture results in lower bond strengths in deep compared to superficial dentin, as immediate bond strengths in deep dentin are generally 30-50% lower than in superficial dentin.[9,10] The relationship between morphology and permeability and how they affect adhesion has been thoroughly discussed in recent reviews.[9,10] We are lacking studies that compare the bond strength durabilities between deep and shallow cavities. However, it may be safe to speculate that lower immediate bond strength and increased risk for hydrolytic degradation of hybrid layer components result with faster and/or more pronounced loss of bond strength with time.

Intact vs Carious Dentin

The minimally invasive dentistry concept aims to limit the cavity preparation to caries removal, although where the limit should be set may be debatable. In any case, most caries excavation methods leave caries-affected and even caries-infected dentin to serve as the bonding substrate.[14]

The immediate bond strengths to caries-affected dentin are commonly 20-50% lower than to sound dentin, and even lower with caries-infected dentin.[9-11,14-17] Caries progression reduces the mineral content, increases porosity, and causes changes in the dentin collagen structure and distribution and noncollagenous protein content.[4] These changes can significantly reduce dentin mechanical properties, such as hardness, stiffness, tensile strength, modulus of elasticity, and shrinkage during drying,[18-21] which make the dentin in and under the hybrid layer more prone to cohesive failures under occlusal forces (Figure 2). Caries demineralization also increases dentin wetness.[19] Lower mineral content allows phosphoric acid or acidic monomers to demineralize the matrix more deeply than in normal dentin, resulting in even more residual water in exposed collagen matrix.[19] The hybrid layers tend to be much thicker and poorly infiltrated, regardless of the bonding system used.[9,10] Together these changes directly affect the strength and durability of the dentin-resin interface.

LOSS OF BOND STRENGTH

In principle, the loss of dentin bond strength can be caused by the hydrolytic degradation of one or both components of the hybrid layer, dentinal collagen and adhesive. Water is needed to maintain dentin collagen scaffold expansion for the resin monomer infiltration.[5] However, excess moisture may cause a phase separation between hydrophobic and hydrophilic monomers, resulting in irregular resin infiltration and forming blisters and voids at the interface. In addition, excess water or other solvents reduce monomer conversion.[8] The consequences of poor resin infiltration and conversion are the reduced durability of the interface, increased enzymatic degradation of the exposed collagen, and the hydrolysis of the poorly polymerized adhesive.[6,9,22,23]

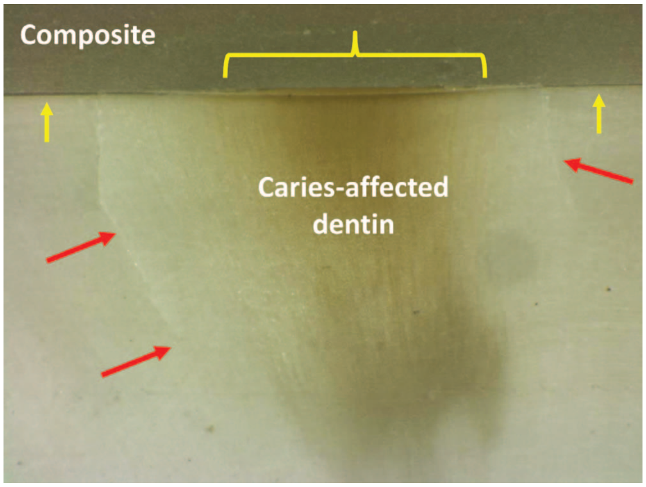

Figure 2. *Composite-dentin interface in caries-affected dentin. At the site of caries-affected dentin, a gap in the restoration-dentin interface is observed (yellow bracket), while the adhesive layer over intact dentin appears normal (yellow arrows). In addition, fracture lines (red arrows) appear surrounding the caries-affected dentin, presumably caused by polymerization shrinkage and stress, impacted by the lower mechanical properties of carious dentin. Stereomicroscope image with reflected light, 32x magnification.*

Hydrolytic Degradation of Adhesives

Dentin wet bonding requires primers or primer/adhesives to contain hydrophilic and ionic monomers to ensure proper hybridization of exposed collagen matrix. 2-Hydroxyethyl methacrylate (HEMA) has many advantages that make it the most commonly used hydrophilic adhesive monomer: it is a small monomer, well solvable in water, ethanol, and/or acetone; it acts as a solvent for hydrophopic monomers; and is relatively biocompatible in polymerized form. An extensive review[22] listed 62 commercially available adhesives, out of which 48 (77%) contained HEMA. Hydrophilicity, however, is a double-sided sword, as, for example, HEMA absorbs water both in the cured and uncured states, which may inhibit polymerization, reduce mechanical properties, and lead to the hydrolytic degradation of polymerized adhesive.[22]

Hydrolytic Degradation of Collagen

Loss of collagen in the hybrid layer was identified about 15 years ago in studies of aged resin-dentin bonds *in vitro*[24] and *in vivo*.[24-27] Ever since, a vast

Table 1: *Matrix Metalloproteinases (MMPs) and Cysteine Cathepsins Detected to Date in Human Dentin. The Proposed Substrates and Function in the Hybrid Layer Are Also Presented for Each Enzyme. It Is Essential to Remember that the Substrates and Functions Are Only Suggestive and Are Based Mainly on Studies with Tissues Other than Dentin. As Studies with Enzyme-specific Inhibitors Are Lacking, the Exact Role and Relative Importance of Each Enzyme Are Not Well Known*

Enzyme	Other Names	Substrates	Function	Reference Nos.
MMP-2	Gelatinase A	Native and partially degraded collagen	Collagen degradation	28-35, 38
MMP-9	Gelatinase B	Native and partially degraded collagen	Collagen degradation	29, 31, 33-35, 38
MMP-8	Collagenase-2	Type I collagen	Collagen degradation	28, 29, 38
MMP-3	Stromelysin-1	Proteoglycans; other noncollagenous proteins	Removal of PGs and PPs	36, 37
MMP-20	Enamelysin	Amelogenin, DSPP	Unknown	38, 42
Cathepsin B	—	Collagen	Collagen degradation	35, 39, 53
Cathepsin K	—	Type I collagen	Collagen degradation	35

Abbreviations: DSPP, dentin sialophosphoprotein; PGs, proteoglycans; PPs, phosphoproteins.

number of morphological and bond strength studies have confirmed time-related loss of collagen in the hybrid layer. Human dentin contains several enzymes that together can degrade dentin collagen matrix proteins, including type I collagen. These enzymes belong to matrix metalloproteinases (MMPs) or cysteine cathepsins (Table 1).[23]

To date, intact human dentin has been shown to contain MMP-8 (collagenase-2),[28,29] MMP-2 and -9 (gelatinases),[28-35] MMP-3 (stromelysin-1),[36,37] MMP-20,[38] cysteine cathepsin B,[35,39] and cysteine cathepsin K.[35] Intense gelatinolytic activity is present in dentinal tubules,[40,41] and MMP-20 is found in dentinal fluid.[42] The physiological roles of these enzymes in dentin are not known, but they have been suggested to participate in peritubular and tertiary dentin formation and in the release of dentinal growth factors during caries, which in turn would regulate pulp defensive reactions.[23,43-47]

Both MMPs and cysteine cathepsins have also been indicated to participate in dentinal caries pathogenesis.[4,35,48-53] MMP-2, -9, and -8 are present in carious dentin,[32,38,45,48] and their activation in pH fluctuations relevant to caries lesions and the role of MMPs in the degradation of dentin collagen have been demonstrated.[48,50] The acidity of the ER[54] and SE[55] adhesives also activates dentinal enzymes. Cysteine cathepsins have also been identified in carious dentin.[35,53] It is important to note that cathepsin K and B and MMP-2 and -9 are dramatically increased in caries-affected dentin compared to intact dentin,[35] and several MMPs increase in dentinal tubules of carious teeth.[42,51,56] MMP inhibitors eliminate human salivary MMP activities[50,57] and reduce caries progression in dentin in animal experiments.[49,50]

METHODS TO IMPROVE DURABILITY

Removal of Caries-infected Dentin

Partial caries removal significantly reduces the risk of pulp exposure and postoperative pulpal symptoms.[58] At the same time it should be recognized that bond strength to carious dentin is weaker and likely more prone to degradation than is bond strength to normal dentin. Bond strength is inversely proportional to caries progression, caries-infected dentin showing the lowest bond strength.[15,16,59,60] Elimination of caries-infected dentin[61] and adequate marginal sealing over sound dental structure[62] are also considered a prerequisite for arresting the caries progression under the restoration and can therefore be seen as minimum requirements for dentin bonding.

Chemical Bonding

Chemical bonding of functional adhesive monomers to hydroxyapatite calcium ions has been suggested to maintain the hybrid layer collagen integrity. These functional monomers, such as 10-methacryloyloxidecyldihydrogen phosphate (10-MDP), 4-methacryloyloexyethyl trimellitic acid (4-MET), or N,N-diethanol p-toluidine (phenyl-P), are usually included in SE adhesives.[2] Mild SE adhesives (with a pH of approximately 2 or higher) leave some hydroxyapatite around the collagen fibrils, believed to keep the resident collagenolytic enzymes inactive and prevent collagen degradation. However, even with 10-MDP (the monomer with the most stable chemical bond) the bond strengths show time-dependent reduction both *in vitro* and *in vivo*.[63-67] Apparently the adhesive penetration and/or polymerization are not sufficient to protect the deepest part of the hybrid layer, which seems to be the weakest area.[63,67,68] If

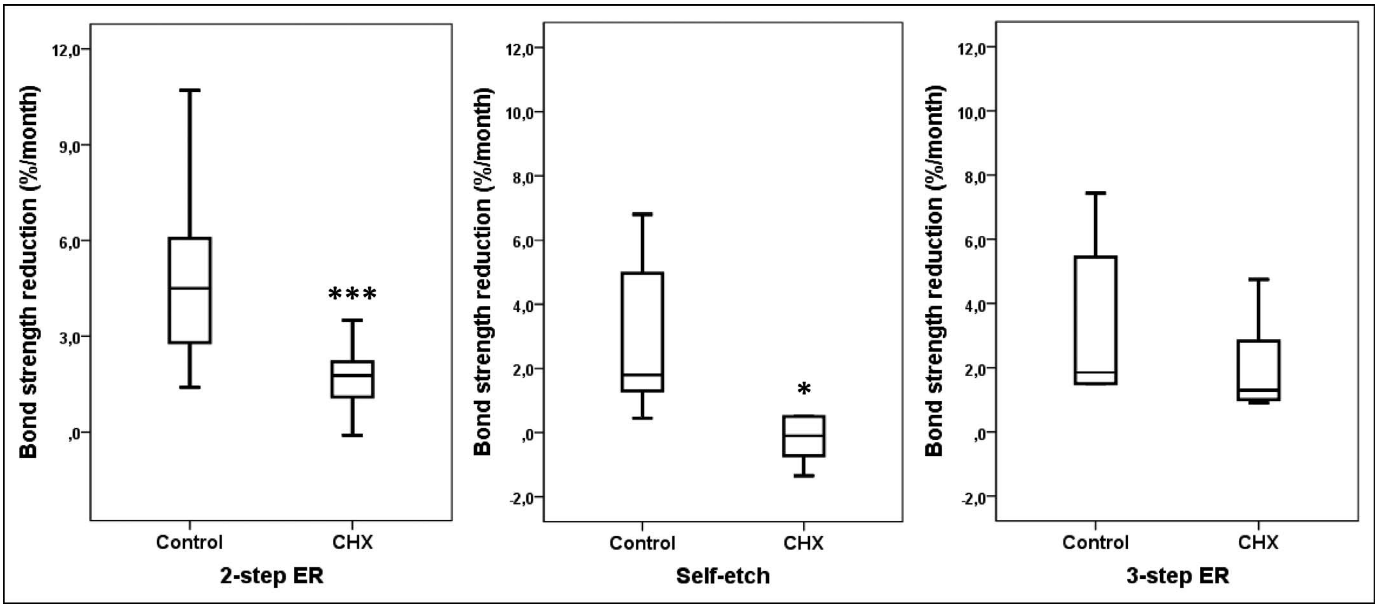

Figure 3. *The comparison of the bond strength reduction (percentage per month) between control and chlorhexidine-treated samples. All microtensile and shear bond strength studies with at least 0.2% chlorhexidine and at least six-month* in vitro *or* in vivo *duration that reported immediate (24 hour to one week) and aged samples bond strengths were included in the analysis. The data included 25 articles, of which 23 involved two-step ER adhesives (77 groups altogether), six studied SE adhesives (15 groups), and five studied three-step ER adhesives (10 groups). ***, significantly different from controls,* $p < 0.001$; **, significantly different from control,* $p < 0.05$.

collagen degradation is not completely eliminated, enzyme inhibition is still needed even with the mild SE adhesives containing functional monomers[6] and may improve bond durability.[66,69]

Enzyme Inhibitors

Numerous approaches based on enzyme inhibition have been tested to slow down or eliminate collagen degradation to preserve bond strength, chlorhexidine being the most popular compound.[6] Chlorhexidine inhibits collagenases and gelatinases present in dentin.[70-73] Several *in vitro*[74-83] and *in vivo*[84-87] studies have demonstrated that chlorhexidine preserves resin-dentin bonds by eliminating or at least delaying collagen degradation in the hybrid layer (Figure 3).

One clinical drawback of the clinical use of chlorhexidine has been the need for a separate priming of dentin before the application of adhesive. In *in vivo* experiments, 15-60 second application of, typically, 0.2-2.0% chlorhexidine on acid-etched dentin before primer/adhesive application has been shown to result in preservation of the hybrid layer and bond strength.[84-87] Incorporation of chlorhexidine into the adhesives is not always a simple task. Chlorhexidine may have a concentration- and material-dependent effect on resin water sorption and solubility,[88,89] degree of conversion,[89,90] and mechanical properties.[89] However, the potential of incorporating chlorhexidine in ethanol-solvated hydrophobic resins has been recognized.[88] In spite of these problems, recent *in vitro* studies have been promising, at least with relatively low chlorhexidine concentrations.[90] Two-step SE adhesive with chlorhexidine added into the primer is effective in inhibiting dentinal MMPs[69] and preventing bond strength loss *in vitro*,[66] and adding chlorhexidine into simplified ER adhesive reduces nanoleakage and time-related loss of bond strength[83] without affecting mechanical properties.[83,91] The first commercially available adhesive system containing 0.2% chlorhexidine (Peak Universal Bond, Ultradent Products Inc, South Jordan, UT, USA) was introduced recently. Whether it is effective in preventing the hybrid layer degradation and time-related loss of bond strength remains to be demonstrated.

Chlorhexidine can also be released from the adhesive in a concentration- and adhesive-dependent manner,[91] which may prove to be beneficial for the controlled enzyme inhibition. Another clinically appealing approach could involve addition of chlorhexidine into the etching acid, as it has been shown to significantly reduce nanoleakage and loss of bond strength for up to two years.[77,79]

Very few studies have addressed the question of the ability of enzyme inhibition to improve long-term bond strength in caries-affected dentin. Komori and others[16] demonstrated with two-step ER adhesive that 2% chlorhexidine significantly improved the six-month bond strength to caries-affected dentin when compared to control treatment: the bond strengths were comparable to the immediate values both with and without caries.[16] Another study[92] demonstrated similar findings with Clearfil SE Bond (Kuraray Co, Ltd, Tokyo, Japan) after two years of aging under simulated pulp pressure. Chlorhexidine has also shown significantly better bond strengths *in vivo* in primary molars for up to 18-20 months when compared to nontreated controls.[93] However, the same study demonstrated time-related loss of bond strength in chlorhexidine-treated teeth as well.[93]

Other MMP Inhibitors—In spite of chlorhexidine's high affinity (substantivity) and stable binding both to mineralized and demineralized dentin,[94,95] the inhibition effect may be lost with time. Because the chlorhexidine molecule is large and water soluble, it may leach out of the hybrid layer, thus limiting its long-term antiproteolytic effect.[93] This reality has driven researchers to look for better alternatives with which to preserve the hybrid layer. Several compounds known to inhibit MMPs have been tested.

Benzalkonium chloride (BAC) is a quaternary ammonium surface-acting agent that has been used as a cavity disinfectant and desensitizer. Commercially available BAC-containing etchants can be used without affecting immediate bond strength to enamel or dentin.[96] BAC also inhibits dentinal MMPs[97-99] and may also improve the durability of dentin bonding when incorporated either into the etchant or the adhesive.[99]

Tetracyclines and their antimicrobially inactive analogs inhibit MMP with their cationic chelating properties and have been shown to inhibit salivary and dentinal MMPs as well.[49,50,57,100] From this group, only minocycline has so far been shown to be potentially useful in adhesive dentistry.[101] Bisphosphonates, such as batimastat, galardin, and zoledronate, also inhibit MMPs by chelating active-site zinc, and zoledronate inhibits carious dentin MMPs.[50] Galardin (0.2 mM) reduces the bond strength loss as effectively as chlorhexidine.[102] Other studies with galardin, batimastat, or SB-3CT (selective gelatinase MMP inhibitor) have failed to confirm the results,[103-105] but in those studies significantly (1:20) lower concentrations were used. This indicates that for effective enzyme inhibition in dentin, higher concentrations are needed than are required with purified recombinant enzymes.

Even though zinc is essential for the activity of MMPs, high zinc concentrations greatly reduce MMP-mediated dentin collagen degradation.[106,107] Zinc-containing adhesives may improve hybrid layer durability and preserve bond strength and may even induce dentin remineralization at the bonded interface.[108-110] The effect is believed to occur via zinc binding to collagen, which protects the cleavage sites of MMPs.[100] However, excessive presence of zinc ions may jeopardize the bonding performance of MDP-containing SE adhesives.[111]

Interestingly, along with tin, zinc has been consistently found under amalgam fillings in dentin considered to represent caries-affected dentin[112,113] and also in artificially demineralized dentin exposed to amalgam.[112] It is tempting to speculate that zinc MMP-inhibiting and/or remineralizing effects may contribute to the resistance of amalgam to secondary caries.

MMP-inhibiting Monomers or Solvents—Another approach with significant clinical interest involves using adhesive monomers that would inhibit collagenolytic enzymes. Polymerizable quaternary ammonium methacrylates (QAMs) are cationic, like chlorhexidine. 12-Methacryloyloxydodecylpyridinium bromide (MDPB) belongs to the QAMs and has been incorporated into SE primer (Clearfil Protect Bond: Kuraray) because of its antimicrobial properties and its ability to copolymerize with adhesive monomers. QAMs inhibit MMPs and demineralized dentin collagen degradation almost completely, MDPB being one of the most effective.[114] Collectively, the *in vitro* and *in vivo* data indicate that MDPB-containing adhesives may be superior in the prevention of bond strength loss, when compared to other SE adhesives.[6,60,64,67,104]

Recently, dimethyl sulfoxide (DMSO) has been identified as a potential solvent with MMP-inhibiting effect.[115] DMSO is a polyfunctional molecule with hydrophilic and hydrophobic groups and an excellent penetration enhancer for medical purposes.[116] It is fully miscible in all solvents and capable of solving many, if not all, monomers used in adhesive dentistry. Because DMSO breaks down the self-associative tendency of water[117] it may reduce water entrapped between the adhesive polymeric chains and enhance the wetting of collagen,[118] allowing better penetration of adhesive into the collagen matrix. These properties may be behind its improved immediate and long-term bond strength; part of the

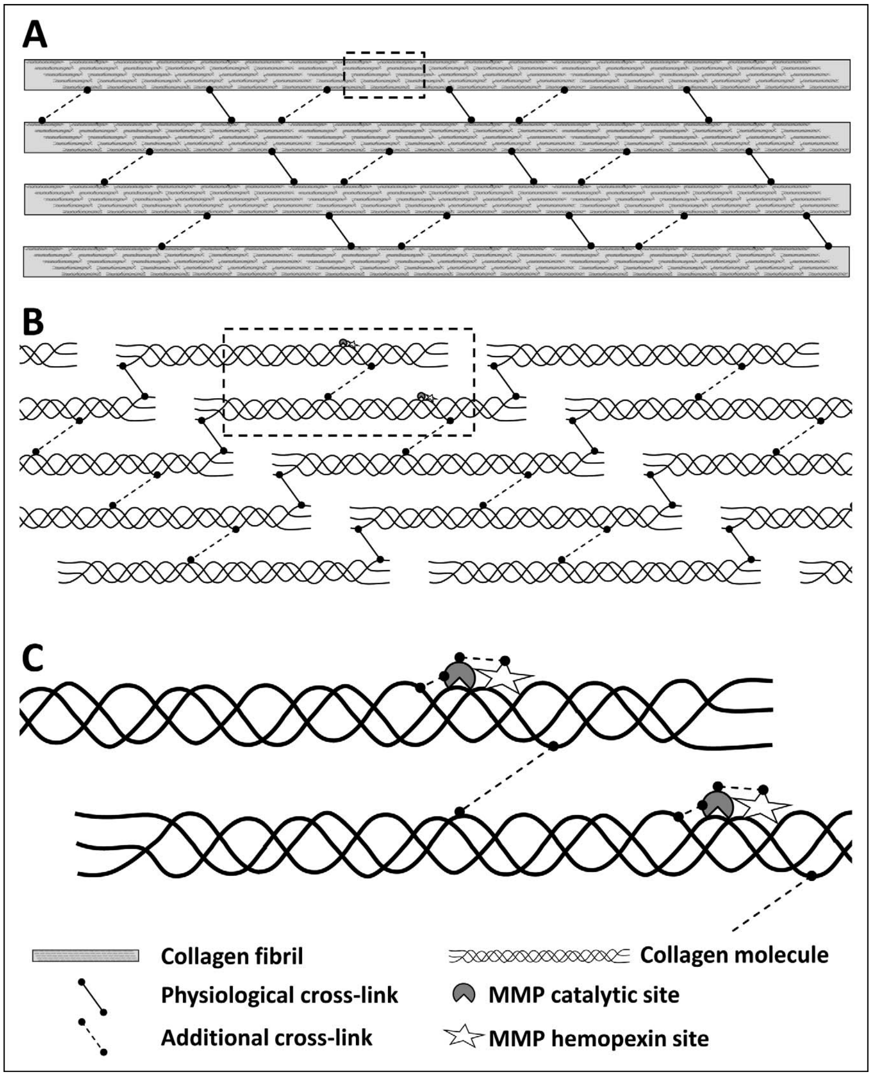

Figure 4. *The proposed mechanism of the effects of an increase in cross-links in dentin matrix (the model adapted from Liu and others[119]). (A) Additional cross-links between collagen microfibrils increase the collagen matrix stiffness and may improve the hydrophobic resin infiltration without the risk of matrix collapse due to drying. (B) More detailed view from the area marked with dashed line in A, demonstrating individual collagen molecules within microfibril. The additional intermolecular cross-links contribute to the increased mechanical properties and increase the matrix resistance against collagen-degrading enzymes. (C) Individual collagen molecules from the marked area in B. Cross-linking may cause conformational changes in the active site, catalytic domain of the protein, and/or binding site of collagenolytic enzymes: all of these may eliminate the enzymes' collagen-degrading capacity.*

effect may be related to its MMP-inhibiting action.[115] Good compatibility with both tissue and adhesive components make DMSO an attractive alternative, as it may be incorporated into the adhesive systems, but more work is needed to confirm the initial promising results.

IMPROVING THE RESISTANCE OF HYBRID LAYER COMPONENTS BY BIOMODIFICATION

Since the pulpal cells cannot remodel or repair lost dental tissue, current restorative therapy aims to replace decayed tissue to restore tooth morphology and function. However, development of biomodification strategies, such as increased collagen cross-linking and biomimetic remineralization, to improve the tissue properties and stability by chemically modifying the tissue offer an interesting approach for adhesive dentistry.[6,21,119,120]

Use of Cross-linkers

The intermolecular and intermicrofibrillar cross-linking is the basis for the stability, strength, and viscoelasticity of dentin collagen matrix. The quantity and type of cross-linking also determines collagen thermal stability and ability to resist biodegradation. Increasing cross-linking of the exposed dentin collagen aims to improve hybrid layer matrix stability[119,120] and may also offer a means by which to increase the use of more hydrophobic adhesives without the risk of collagen matrix collapse during adhesive application (Figure 4).

Studies of cross-linkers have primarily focused on their ability to enhance the mechanical properties of demineralized dentin.[120] In addition to stiffening, cross-linking can also affect enzymatic degradation by allosteric silencing of collagenolytic enzymes (Figure 4) or by altering the enzyme binding site in the collagen molecule.[119-124] The cross-linkers tested include synthetic cross-linkers such as carbodiimide and glutaraldehyde, physical (photo-oxidative) cross-linking with riboflavin, and naturally occurring compounds such as genipin and proanthocyanidins.[120] Here we will concentrate on the studies dealing with the preservation of the hybrid layer; for detailed information about cross-linkers in dentin biomodification, the reader is referred to a recent extensive review.[120]

Glutaraldehyde is a widely known cross-linker that has been used in dental products, but its cytotoxicity seriously limits its clinical use.[6,120] Carbodiimide hydrochloride has very low cytotoxicity but may have limited cross-linking capacity.[120] It has been demonstrated to eliminate collagen degradation and preserve bond strength *in vitro*,[125] even though the time needed for the effect may still be too long for clinical practice.[6] Dentin treatment with carbodiimide is effective in inhibiting dentinal MMP activity alone or mixed with HEMA, which indicates that it could be added to adhesive primers.[124] Proanthocyanidins are also effective.[126,127] An increase in immediate dentin bond strength may be achievable even with shorter treatment times,[128,129] and improved durability of long-term bond strength has also been indicated.[81,130] Riboflavin has also been successfully tested, but the need for ultraviolet light or separate cross-linker light curing[131,132] reduces its clinical acceptability.

Biomimetic Remineralization

Biological mineralization of all hard tissues is a progressive dehydration process; with the increasing mineral content the water content of the collagen matrix decreases correspondingly to maintain a constant volume.[21,119] In dentin bonding, resin adhesive is incapable of dehydrating the collagen matrix sufficiently,[1,5,6,133,134] leaving behind water that will allow hydrolysis of the hybrid layer components. Biomimetic remineralization mimics the progressive dehydration of natural biomineralization by replacing matrix water with apatite crystallites (for a comprehensive review, see Niu and others[21]). In the hybrid layer, replacing water with minerals would increase mechanical properties and inhibit water-related hydrolysis.

In biomimetic remineralization of the hybrid layer, polyanions (eg, polyacrylic acid or polyaspartic acid) bind to collagen and serve as analogs of dentin phosphoproteins that regulate physiological mineralization, allowing calcium binding and promoting apatite nucleation. The hybrid layer is covered with a "therapeutic" composite containing amorphous calcium phosphate as a source for apatite. *In vitro* studies indicate that biomimetic remineralization has great potential for remineralizing hybrid layers or caries-like dentin.[21] These studies have also demonstrated the preservation of the mechanical properties of the hybrid layer[135] and bond strength[136] with time.

Even if biomimetic remineralization strategy has great potential—and should perhaps be the ultimate goal of research—in preventing the loss of hybrid layer integrity, it must be realized that to date the strategy is still at the proof-of-concept stage. Development of clinically applicable materials that would contain and release the critical components of the process (at least calcium and phosphate source and biomimetic analogs) involves considerable challenges.[6,21,119]

ELIMINATION OF HYDROPHILIC ADHESIVES

In attempts to reduce the hydrolytic degradation of adhesive, less hydrophilic HEMA-free adhesives have been created.[8] However, the solvation effect of HEMA is also lost. When other solvents, such as acetone or ethanol, evaporate, water tends to separate adhesive components, making these adhesives prone to phase separation.[137,138] The resulting water blisters may lower the immediate bond strength. The studies examining the durability of bond strength with HEMA-free adhesives are limited and the results are conflicting, but generally, loss of bond strength seems to occur.[139-141] Extended evaporation (air drying) of the adhesive layer may reduce the blister formation.[137,138,142]

Ethanol-wet Bonding

Preventing hybrid layer degradation with the use of MMP-inhibitors or MMP-inhibitor–conjugated resin monomers will continue to be the predominant method for extending the longevity of resin-dentin bonds until a more proactive solution becomes clinically available. However, a water-rich zone rich in polymerized or unpolymerized hydrophilic monomers along the resin-dentin interface continues to be a problem. Even if collagen matrix can be preserved, loss of integrity through the degradation of adhesive component will remain.[143] This may be the reason for the slow loss of bond strength even when enzyme inhibition has been successful.

Ethanol-wet bonding aims to use ethanol to dehydrate demineralized dentin matrices and to assist the infiltration of more hydrophobic monomers into dentin.[1,5,6] Infiltration of hydrophobic monomers decreases water sorption and solubility and resin plasticization and may prevent or at least reduce enzymatic hydrolysis of collagen.[144-147] Together, these would lead to improved bond durability.[1,6] Currently, however, technique sensitivity and generally long treatment times prohibit ethanol-wet bonding in clinical settings, and more user-friendly and reproducible techniques or materials need to be developed for everyday use.[6,9]

CAN DENTIN BONDING BE MADE TO LAST?—CONCLUSIONS

The overall clinical success of composite restorations is multifactorial and therefore is unlikely to be predicted by even a battery of *in vitro* test methods. Only limited evidence exists to correlate marginal quality and bond strength in the laboratory with the clinical performance of bonded dental composites.[148] However, it is clear that rapid deterioration of dentin bonding does not improve the restoration longevity. It is equally clear that it is possible to prevent or at least significantly slow down hybrid layer degradation.

As discussed above, carious dentin is not necessarily an ideal substrate for strong and durable hybrid layer formation. Therefore, careful removal of at least caries-infected (soft) dentin and removal of carious dentin close to the cavity margins are minimum requirements.

Despite the development of faster and more simple adhesives, conventional three-step ER and two-step SE adhesives are still considered the most reliable alternatives and the benchmark for dental adhesion in clinical practice.[1,2,8,9,133,149] Addition of a separate hydrophobic adhesive resin after the hydrophilic primer makes the interface more hydrophobic and seals it more effectively, resulting in a more durable bond than is obtained with the simplified two-step ER or one-step SE adhesives. Although enamel-bonded restoration margins cannot prevent the degradation of resin-dentin hybrid layer *in vivo*,[84,85] a proper sealing of enamel margins should result in a more favorable clinical outcome.[134,149] Since milder SE adhesives in particular do not necessarily provide sufficient enamel etching, separate acid-etching of enamel margins is recommended with SE adhesives.[2,150]

Inhibition of collagenolytic enzymes with chlorhexidine has the best *in vitro* and *in vivo* evidence in terms of clinical use as well. Although chlorhexidine may not be perfect, and although long-term clinical performance studies are still lacking, no adverse effects have been reported either. Some manufacturers already recommend application of chlorhexidine as an optional step after acid-etching or before application of SE primer. It is safe to conclude that until other approaches have been proven safe and at least equally effective, chlorhexidine (either via separate application or as incorporated into adhesive system) can and should be used.[6,133]

The techniques and materials that allow the use of hydrophobic adhesives offer another attractive alternative, but this approach may require new monomers with different chemistry. Combining cross-linkers with ethanol-wet bonding, DMSO, or a corresponding agent may offer an easier approach. If cross-linkers can be used to stiffen the exposed collagen matrix sufficiently to prevent shrinkage during rapid and complete removal of water, it should be possible to infiltrate the matrix with hydrophobic monomers. Increased resistance and enzyme inhibition with cross-linkers, ethanol, and DMSO together with complete encapsulation of collagen should efficiently prevent collagen matrix degradation, and an absence or minimal amount of hydrophilic monomers would help to preserve the adhesive component of the hybrid layer. Biomimetic remineralization—returning the hybrid layer collagen to, or close to, its original mineralized state—should, however, remain the ultimate goal.

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Conflict of Interest

The author has no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Clinical Technique

A Restorative Approach for Class II Resin Composite Restorations: A Two-Year Follow-up

MJMC Santos

Clinical Relevance

Successful resin composite restorations can be achieved when a careful restorative technique is employed. Use of a sectional matrix band and elastic ring helps achieve a tight proximal contact, and the centripetal restorative technique can help to obtain contour and anatomy, minimizing the use of rotary instruments during the finishing procedures.

SUMMARY

This clinical report describes a restorative technique used to replace two Class II resin composite restorations on the upper premolars. A sectional matrix band was used in conjunction with an elastic ring (Composi-Tight) to obtain tight proximal contact. A nanofilled resin composite (Filtek Supreme Ultra) was incrementally applied using oblique layers to reduce the C-factor, each layer being no more than 2 mm thick, and then light cured for 20 seconds with a light-emitting diode lamp (EliparFreeLight 2 LED Curing Light) with a power density of 660 mW/cm^2. A centripetal technique was used to restore the lost tooth structure from the periphery toward the center of the cavity in order to achieve a better contour and anatomy with less excess, thereby minimizing the use of rotary instruments during the finishing procedures. Finally, the resin composite restorations were finished and polished, and a surface sealer (Perma Seal) was applied to fill small gaps and defects that may have been present on the surfaces and margins of the restorations after the finishing and polishing procedures.

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INTRODUCTION

Resin composites have been used largely as direct restorative materials because of their toothlike appearance, low cost, long working time/command cure, and acceptable clinical behavior.[1,2]

Among the disadvantages, increased marginal discoloration and reduced marginal adaptation have been reported in several clinical studies.[3-5] Breakdown of the adhesive bond poses a challenge to the longevity of composite restorations as microleakage can lead to secondary caries.[5,6]

Clinical studies have suggested that resin composite restorations present better results in small to moderate-sized cavities.[1,2,4] The performance of these restorations seems to be more successful in premolars than in molars, with fracture and secondary caries being the most common reasons for failure.[6-9] Also, the presence of enamel along the cavity margins has been considered an ideal condition because it allows for a peripheral resin-enamel seal that retards ingress of external fluids and bacteria. Once water and bacteria diffuse along the resin-dentin interface, they accelerate the degradation of the adhesive interface.[10]

Another important problem faced by clinicians when performing Class II composite restorations is to reestablish proximal contact. The lack of condensability of composite materials allied to the thickness of the matrix band poses a challenge when trying to achieve adequate interproximal contact. Several instruments and techniques have been developed in an attempt to solve this problem.[11,12] Among them, the use of pre-polymerized resin composite balls, pre-contoured instruments, and sectional matrices with elastic rings have been reported.[12-14] The aim of this report is to present a clinical case in which successful Class II restorations were achieved using pre-contoured sectional matrices and a separation ring to obtain a tight proximal contact. At a two-year evaluation, the restorations presented very satisfactory clinical behavior.

CASE REPORT

A 28-year-old man in excellent oral health was referred for an oral examination at the dental clinic of Schulich Medicine & Dentistry, Western University, London, Canada. His chief complaint was related to sensitivity in the left maxillary premolar region when ingesting sweet food. Although no recurrent caries was visualized on the bite-wing radiographs, deficient marginal adaptation was clinically detected on the old resin composite restorations of teeth 14 and 15 (Figure 1). Because of the conservative size of the cavity preparation and the patient's good oral hygiene allied to the esthetic requirement, it was agreed to replace these defective resin composite restorations with the same material.

Isolation was performed using a rubber dam. The old composite restorations were removed, and the preparations were refined with a No. 245 carbide bur. Bevels were placed at the facial and lingual walls of the proximal box using a diamond needle bur (DET-CEF, Brasseler, Quebec, Canada). Unsupported enamel at the gingival margins was finished with gingival margin trimmers (Hu-Friedy Mfg Co, Chicago, IL, USA) (Figure 2).

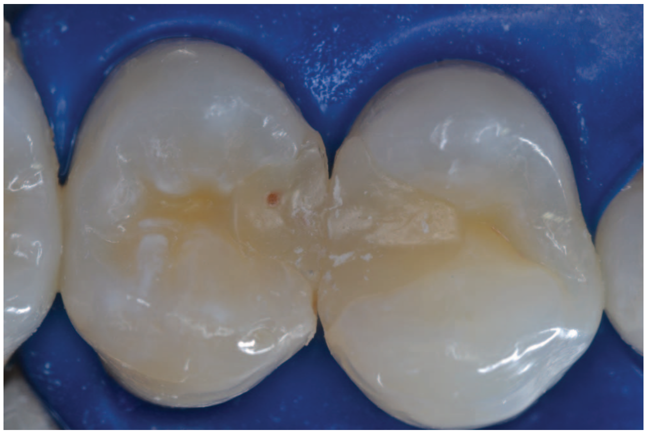

Figure 1. *Initial aspect of the defective resin composite restorations. Note the presence of unsatisfactory contour and marginal gap at the mesiofacial margin of tooth 15.*

A sectional pre-contoured matrix system with an elastic ring (Composi-Tight ring, Garrison Dental Solutions, Spring Lake, MI, USA) was applied, and a ball burnisher was used to verify contact with the adjacent tooth. The restorative procedures can be visualized step-by step in Figure 3a-h.

The cavity preparation was conditioned with 37% phosphoric acid for 15 seconds in the dentin layer and 30 seconds in the enamel layer, after which the cavity was rinsed and gently dried with an air syringe, leaving a slightly moist surface. The dentin bonding agent was applied (Single Bond adhesive, 3M/ESPE, St Paul, MN, USA), gently air dried to evaporate the solvent, and light cured for 10 seconds with a light-emitting diode lamp (EliparFreeLight 2 LED Curing Light, 3M/ESPE) with a power density

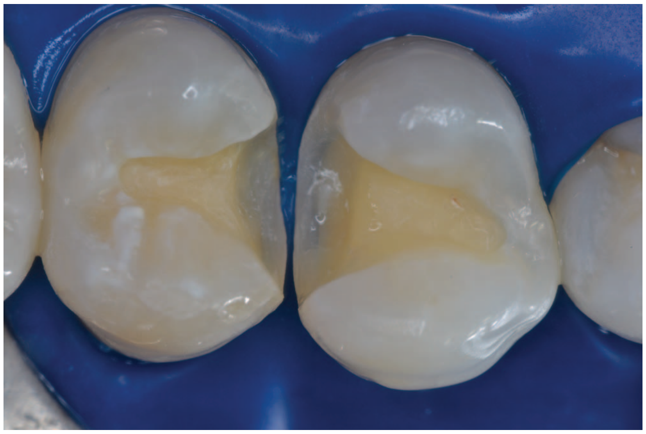

Figure 2. *Restorations were removed and the preparations were refined with a No. 245 carbide bur. Note the presence of enamel around all margins of the cavity preparation. Obtuse angles were developed at the proximal margins to increase the number of enamel rods exposed for the acid conditioning.*

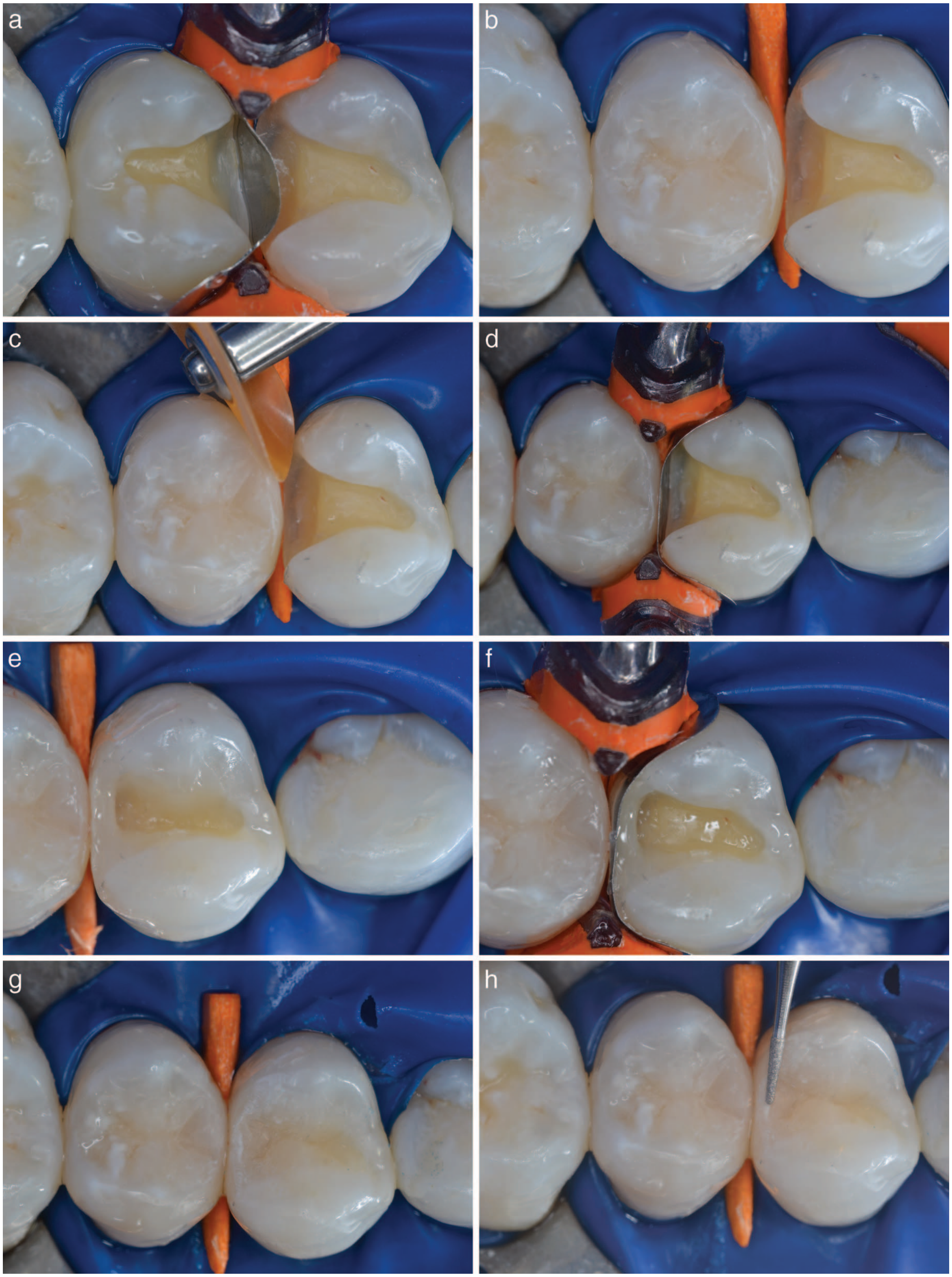

Figure 3. *Restorative procedure. (a): Sectional matrix, wedge, and elastic ring were positioned on tooth 15. (b): Aspect of the final restoration. (c): Proximal and occlusal embrasures were refined with fine disks before starting the restorative procedure on the neighboring tooth. (d): Sectional matrix, wedge, and elastic ring were positioned on tooth 14. (e): Resin composite was applied from the periphery to the center of the cavity (centripetal technique). (f): After proximal contour was reestablished, the matrix was removed to promote better access and visualization to the occlusal box. (g): Aspect of the restoration immediately after its conclusion. (h): A diamond bur was used to refine contour and remove any small excess.*

of 660 mW/cm^2. The nanofilled resin composite (Filtek Supreme Ultra, 3M/ESPE) was incrementally applied in oblique layers no more than 2 mm thick to reduce the C-factor, and then light cured for 20 seconds. The resin composite was applied from the periphery to the center of the cavity preparation in order to first reestablish the proximal contact. After proximal contact was reestablished, the matrix band and elastic ring were removed, and the occlusal box was restored. The centripetal technique has the advantage of transforming the Class II into a Class I, and facilitating visualization and access because the matrix band is removed immediately after the proximal box is restored.

Finishing and polishing procedures were accomplished with the use of a diamond bur (DET-CEF, Brasseler) followed by rubber points (Pogo Points, Dentsply Caulk, Milford, DE, USA). Proximal and occlusal embrasures were refined with fine disks (Sof-lex Finishing/Polishing Kit, 3M/ESPE). After polishing, a surface-penetrating sealant was applied to each restored tooth (Perma Seal, Ultradent, South Jordan, UT, USA). The resin composite surfaces, including the margins, were etched with 35% phosphoric acid for 5 seconds, rinsed and dried. A thin layer of a surface sealer was then rubbed into the surfaces, gently air thinned, and light-cured for 20 seconds (Figure 4).

At one and two years after treatment, the restorations were checked. The patient was satisfied with the result (Figure 5).

DISCUSSION

Although resin composite materials are considered easy to handle, reestablishing proximal contact is sometimes a challenging procedure, especially when the clinician is placing large Class II restorations. Unlike amalgam, which can be laterally condensed to obtain an optimal proximal contact, esthetic composite materials depend entirely on the contour and position of the matrix and wedge.[11,15] The lack of condensability because of the visco-elastic properties of the composite materials makes reestablishment of proximal contact more difficult and requires much care in adapting the matrix and wedge.

Different types of matrix systems have been specially developed for use with composite restorations. Compared with plastic matrices, metal matrices are considered easier to install, maintain their shape better, are thinner, and can be burnished to the adjacent tooth, so the interproximal contacts can be more easily developed.[16]

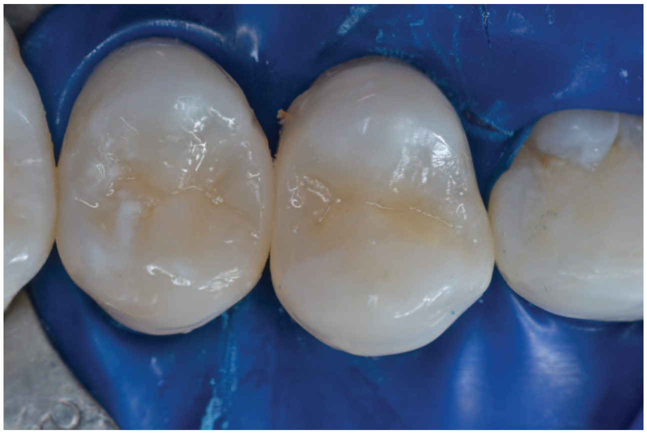

Figure 4. *Final aspect of the Class II restorations after application of the surface sealer.*

More recently, sectional matrices, which feature a short piece of steel matrix that is designed for single proximal-surface restorations, were designed with the intention of simplifying the matrix placement procedure. The great advantage of this system is the presence of an elastic ring that holds the contoured matrix in place. These rings provide progressive

Figure 5. *Aspect of the restorations. (a): After one-year clinical evaluation. (b): After two-year clinical evaluation.*

tooth separation, resulting in an efficient contact.[11,13,15]

Although the use of pre-contoured instruments can help reestablish interproximal contact when circumferential matrices are used, previous studies have shown that the greatest increase in tightness is achieved when elastic rings are used.[11-15] The separation promoted by the rings can compensate for the thickness of the matrix band and allows for good adaptation of the composite material to the neighboring tooth.

To reduce the stress generated during the polymerization contraction, oblique increments contacting the maximum of two walls were used to reduce the C-factor.[17] Additionally, the incremental placement technique is necessary to ensure full curing of the entire bulk of composite and to facilitate the anatomic buildup of the restoration. Increments were applied to replace one cusp at a time. The uncured composite was contoured to the final anatomy of the cusp and then light-cured. This procedure allowed for the achievement of an ideal contour without the need to use the bur extensively during the finishing procedure. The use of the centripetal technique also contributed to the better access of the occlusal box once the matrix and ring were removed, allowing better visualization and positioning when replacing the missing dental structures at the cusps. This technique was first described in 1994 by Bichacho[18] and was intended to restore the lost tooth structure from the periphery toward the center of the cavity in order to achieve a better contour and anatomy with less excess, thereby minimizing the use of rotary instruments during the finishing procedures.

The use of rotary instruments when contouring and finishing the composite restorations' surfaces may create defects on the surface.[19] The use of surface sealers has been advocated to fill small gaps and defects that may be present on the surfaces and margins of restorations after the finishing and polishing procedures.[19,20] Surface sealers are light-cured materials that present greater fluidity and penetration capacity. The surface sealant can seal the margins and any microscopic gaps or defects in the surface, promoting better marginal adaptation and extending the restoration's longevity by protecting the underlying composite and delaying its exposure to the oral environment.[20-22] Some *in vitro* studies have also shown reduced wear[23] and decreased microleakage of resin composite restorations that have been sealed with resin surface sealers.[21,22]

In the present case, both restorations were conditioned with a 35% phosphoric acid for 5 seconds to facilitate impregnation of a fluid resin with a high-penetrating capacity to fill possible marginal discrepancies that might have been generated during the finishing and polishing procedures.

CONCLUSION

Successful resin composite restorations can be achieved once the characteristics and limitations of these materials are understood and taken into consideration. Because of the peculiar features of resin composite materials, such as the stress generated as a result of polymerization shrinkage, viscoelastic properties that preclude proper condensation, thickness/cure ratio, and technique sensitivity of the bonding protocol, a careful restorative technique should be used. In conclusion, all phases involved in the restorative procedure should be meticulously implemented to ensure the success of the resin composite restorations.

Conflict of Interest

The author of this manuscript certifies that there is no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Color Masking of Developmental Enamel Defects: A Case Series

CRG Torres • AB Borges

Clinical Relevance

White developmental enamel lesions can be successfully masked using the resin infiltration technique, resulting in a satisfactory esthetic appearance of affected teeth.

SUMMARY

Developmental defects involving color alteration of enamel frequently compromise the esthetic appearance of the tooth. The resin infiltration technique represents an alternative treatment for color masking of these lesions and uniformization of tooth color. This technique is considered relatively simple and microinvasive, since only a minimal portion of enamel is removed. This article illustrates the color-masking effect with resin infiltration of fluorosis and traumatic hypomineralization lesions with a case series. The final esthetic outcomes demonstrated the ability of the resin infiltrant to mask the color of white developmental defect lesions, resulting in satisfactory clinical esthetic improvements. However, in more severe cases, the color-masking effect was not complete.

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INTRODUCTION

Because of patients' increasing desire for esthetic smiles, dentists are more often required to treat abnormalities in tooth color. White color alterations can occur as consequences of post- or pre-eruptive damage.[1] White spot lesions are posteruptive alterations that take place when demineralization overcomes the remineralization, within the dynamics of the carious process. These lesions are characterized by a loss of mineral beneath a pseudo-intact surface layer, and these increased porosities present in the lesion subsurface, resulting in the whitish appearance of enamel.[2,3]

The most frequent pre-eruptive whitish enamel lesions are fluorosis, traumatic hypomineralization, and molar-incisor hypomineralization (MIH). Fluorosis is caused by excessive long-term ingestion of fluoride during tooth development, characterized histopathologically by a relatively well-mineralized outer surface layer, beneath which a diffuse hypomineralization, or porosity in subsurface enamel, occurs.[4,5] Fluoride has been shown to affect predominantly the maturation stage of enamel formation, and the hypomineralization increases with the fluoride exposure, reflecting in different degrees of severity, with increasing clinical effects in the esthetic appearance of the teeth.[6] The clinical manifestation of milder forms of fluorosis-induced enamel changes appears as narrow white lines following the perikymata, cuspal snowcapping, and a snowflaking appearance without defined borders.[7,8] The main characteristic of this damage is the symmetrical involvement of homologous teeth and often the involvement of several groups of teeth.[1]

The traumatic hypomineralized lesion is a consequence of a periodontal disturbance involving the primary tooth, affected by luxation (displacement) injuries, during the mineralization phase of the permanent tooth.[1] In addition, periapical infection of a deciduous tooth can also affect the germ of its permanent successor.[9] The degree of damage to the permanent successor depends on the stage of crown formation, with clinical appearance varying in extension, limits, shape, location, and color. These discolorations are the result of an accelerated deposition of minerals and are generally punctiform, well defined, present in the incisal third of tooth crowns, and often limited to one tooth.[1,10] The histological characteristic of these lesions is similar to white spot demineralizations and fluorosis, since it involves subsurface hypomineralization under a relatively well-mineralized surface.[1,7]

Molar incisor hypomineralization is defined as hypomineralization of systemic origin in at least one of the four permanent first molars, frequently associated with affected incisors.[11] The lesions often present a surface layer with increased mineral content, but the nature of MIH-affected enamel is highly variable regarding mineral content, hardness, and porosity.[12] In MIH defects, hypomineralization begins at the enamel-dentin junction and not at the surface of the enamel, and in mild cases, it is often situated in the inner third of the thickness of enamel.[1]

All of these conditions result from a reduction of the mineral phase, altering the chemical composition of enamel and, consequently, its optical characteristics. However, in each case, the hypomineralization assumes distinct topographical forms.[1] The correct diagnosis is essential to determine the most adequate treatment for each particular discoloration, which involves bleaching, microabrasion, resin infiltration, and, in the most severe cases, restorations.

The resin infiltration technique was introduced as an alternative therapeutic approach based on the penetration of a low-viscosity resin, with high penetration coefficient, into the intercrystalline spaces of the porous lesion, rehardening the demineralizing tissue and inhibiting further progression of the enamel caries lesions.[13,14] In addition, as the subsurface lesions are filled with resin, a positive effect regarding color masking of the whitish lesions can be observed; therefore, this technique has been used in esthetically compromised areas.[3,15]

These masking effects were initially observed in enamel carious lesions, with satisfactory outcomes demonstrated in both *in vitro* investigations[16,17] and clinical cases.[3,18] This motivated the application of the resin infiltration technique as an attempt to mask white lesions resulting from developmental defects, such as fluorosis and traumatic hypomineralization injuries.[19]

The objective of this clinical case series is to describe the resin infiltration technique for masking developmental enamel lesions presenting white discolorations, illustrating the clinical steps related to the technique and the immediate esthetic outcomes.

CLINICAL CASES REPORT

This article presents a case series of five patients aged between 17 and 26 years who exhibited enamel white discolorations in esthetically compromised tooth areas. Anamnesis and clinical assessment were performed to determine the etiology of discolorations. All patients or parents signed an informed consent authorizing the treatments and use of images. The treatment decision was based on minimal intervention dentistry, using the resin infiltration technique with low-viscosity resin (Icon, DMG, Hamburg, Germany) as an attempt to mask these lesions.

Cases 1 and 2 (Figures 1 and 2) were diagnosed as very mild and mild-to-moderate fluorosis. Case 3 (Figure 3) was considered fluorosis associated with hypomineralized and hypocalcified defects. Cases 4 and 5 (Figures 4 and 5) were classified as hypomineralized spots resulting from injuries to the permanent incisor as a consequence of trauma to the primary tooth.

When lesions were close to the gingival margin, a conventional rubber dam with ligatures was used to protect the oral soft tissues, deflect the gingival tissue, expose the cervical portion of the tooth, and provide a clean and dry working field. On the other hand, when no deflection of the gingival tissue was necessary, a resinous gingival barrier (liquid rubber dam) was used.

After cleaning with prophylaxis pumice, the affected areas were etched with 15% hydrochloric acid (Icon-etch) for two minutes and then washed with water spray for at least 30 seconds. At this time, the lesions were assessed for color alteration, and if

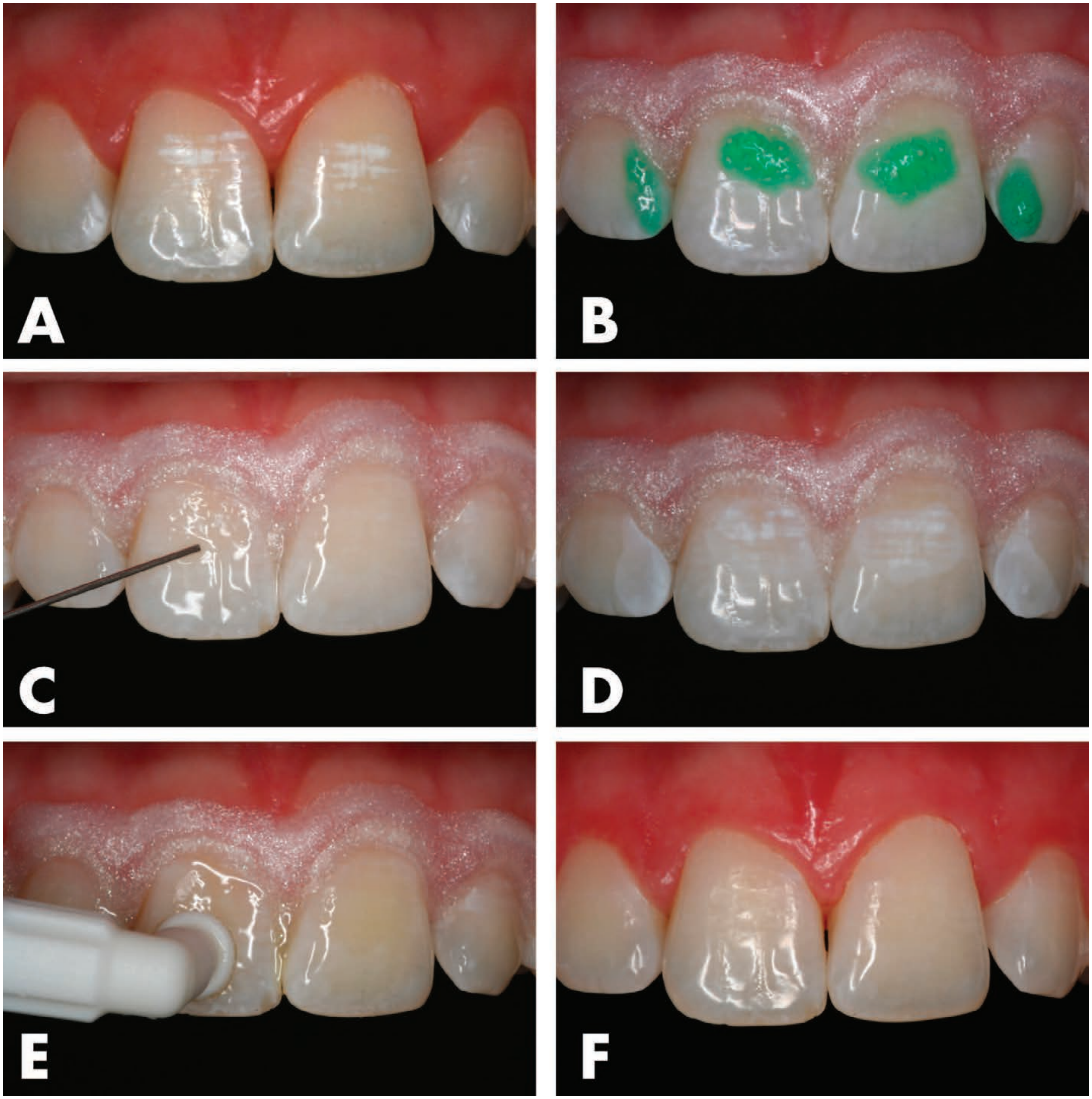

Figure 1. *Case 1, fluorosis TF 1. (A): Preoperative view, with white color alteration in the middle portion of the central incisors, corresponding to the position of the perikymata and a slight snowcapping of mesial angles of lateral incisors. (B): Resinous gingival barrier in position and 15% hydrochloric acid application (three applications of two minutes). (C): Ethanol application, with preview of the masking effect. (D): Enamel appearance after drying. (E): Infiltration of the resin. (F): Postoperative view with improved esthetic appearance.*

no visual color change was obtained with water, the etchant was applied again for an additional two minutes, until some color alteration could be observed at the wet eroded surface. The surface was then air dried, and ethanol (Icon-dry) was applied for 30 seconds to maximize the water removal inside the lesions. The lesions were air dried again, and the surfaces exhibited a chalky white appearance. The resin infiltrant (Icon) was then applied on the lesion surface, and it was allowed to penetrate for three minutes. Excess resin was removed using a blow of air, and light curing was performed for 40 seconds.

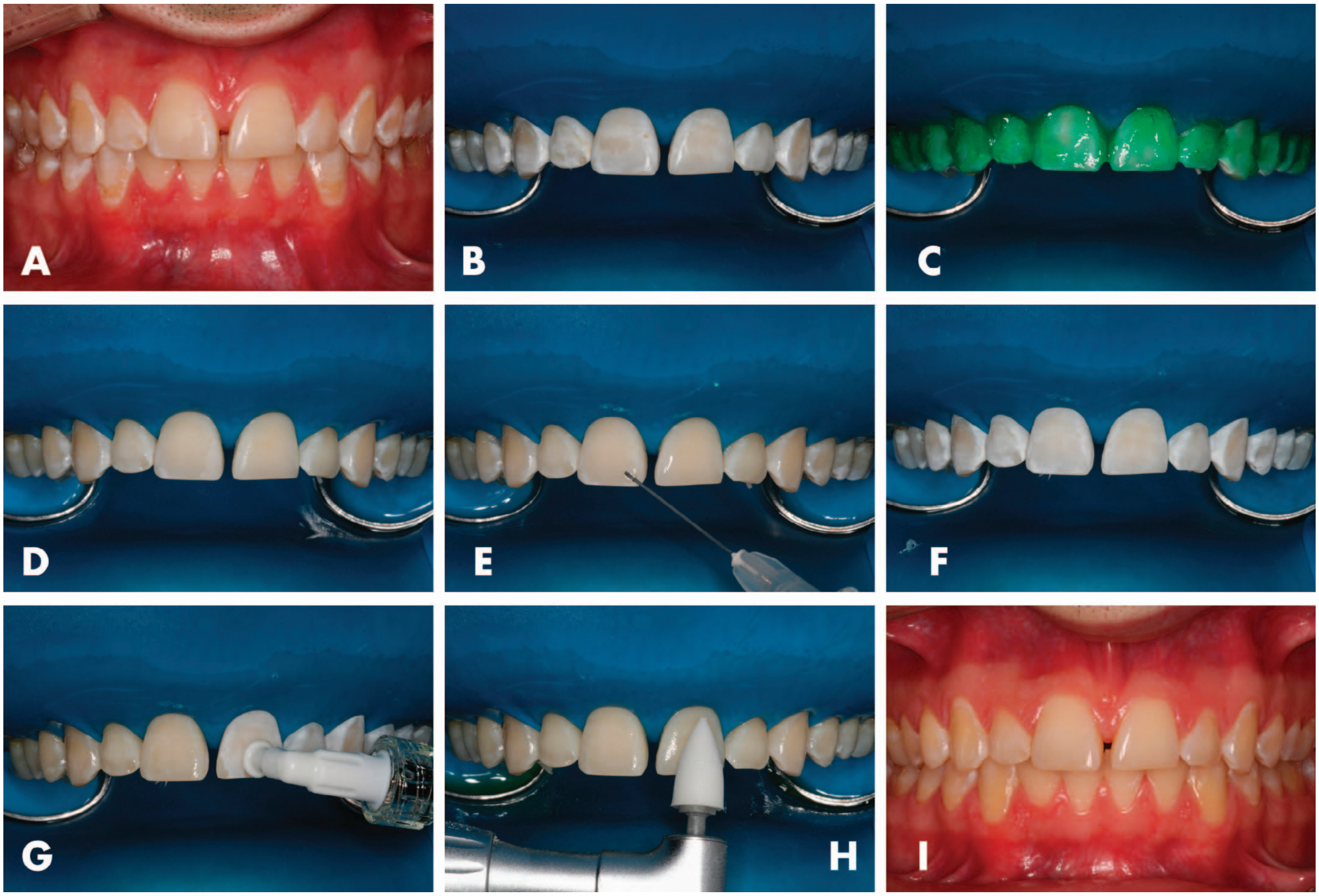

Figure 2. *Case 2, fluorosis TF 3. (A): Preoperative view, with irregular cloudy white areas spread over the crowns. (B): Rubber dam positioned in the superior arch. Ligatures were performed in all affected teeth with the knot in the lingual side. (C): Fifteen percent hydrochloric acid application (two minutes in all teeth and two additional applications of two minutes in the most affected areas) (D): Wet eroded surface, exhibiting a preview of the masking effect (E): Ethanol application. (F): Enamel appearance after drying. (G): Infiltration of the low-viscosity resin. (H): Polishing procedures. (I): The same technique was repeated in the lower arch, postoperative view with improved esthetic appearance.*

The resin infiltrant application was repeated for one minute, followed by light curing for 40 seconds. The surfaces were polished using fine-grained abrasive flexible discs, rubber points, and finishing strips, depending on the treated area. An immediate esthetic improvement, with partial or total color masking, could be observed after treatment. The final pictures were obtained one week after the end of the treatment, allowing rehydration of the teeth and gingival tissue repair.

DISCUSSION

Contemporary dentistry often endeavors to offer clinical solutions for dental problems with minimal involvement of sound tooth tissues. Different approaches have been used to improve the esthetic appearance of white hypomineralization lesions. Tooth-whitening techniques have been employed, with the aim of bleaching regular enamel, camouflaging the white-involved areas, and making the tooth color more uniform. Nevertheless, the results are not always satisfactory, and in many cases, microabrasion with pumice and hydrochloric acid needs to be performed. Enamel microabrasion can produce acceptable esthetic improvement in shallow lesions,[20] and although the amount of enamel loss is related to the acid type and concentration, abrasive particles, duration and number of applications, and

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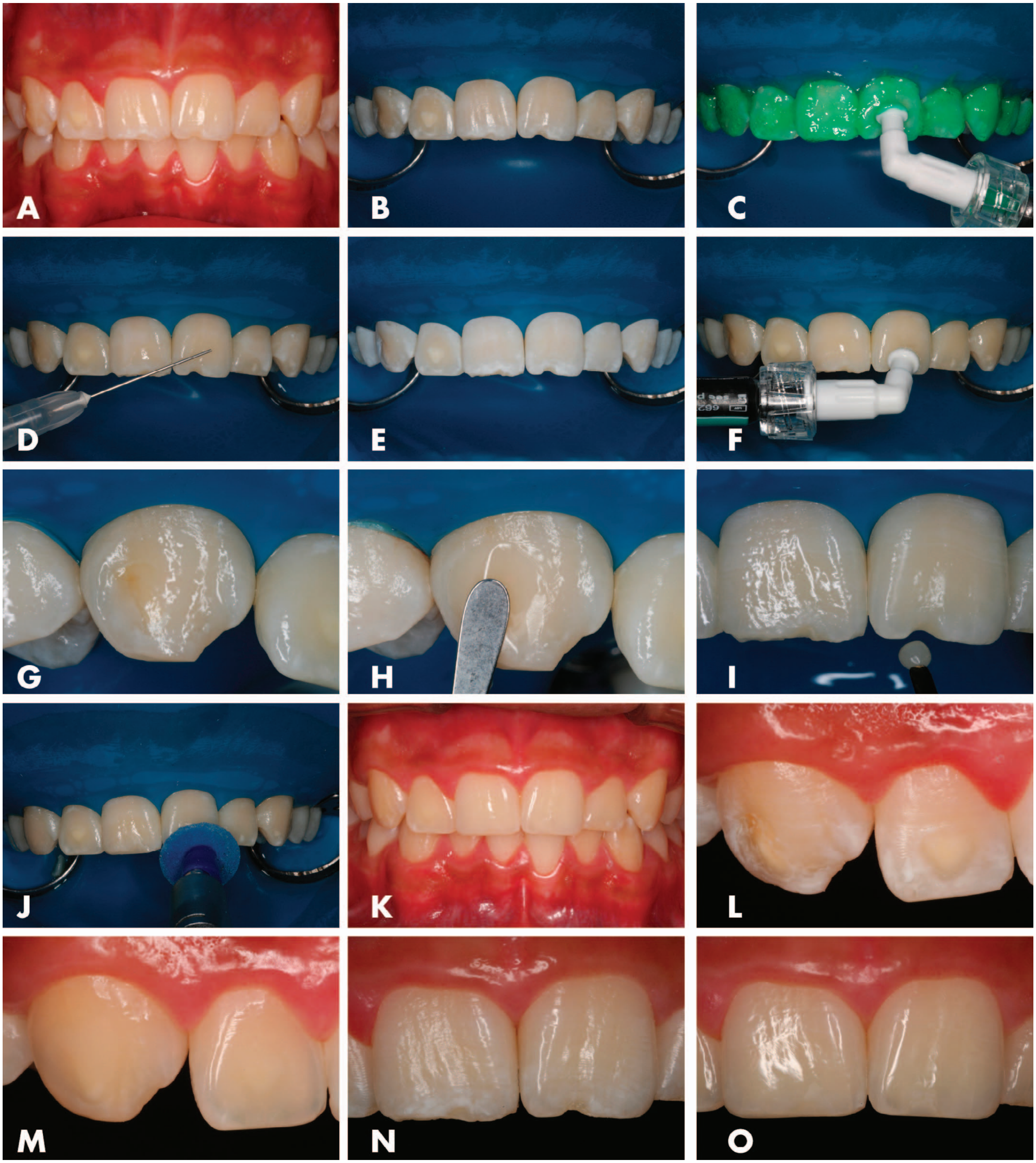
Figure 3. *Case 3, fluorosis TF 1 associated with hypoplasic defects. (A): Preoperative view, with very mild fluorosis distributed through the teeth, hypocalcificacion defect in the central part of the right lateral incisor, and hypoplasic defects in the superior right canine and incisal borders of central*



maxillary incisors. (B): Rubber dam in place. (C): Fifteen percent hydrochloric acid application for two minutes in the labial face of the teeth; two additional applications were performed in the lateral incisor and canine for two minutes each. (D): Ethanol application, showing a preview of the masking effect. (E): Enamel appearance after drying. (F): Resin infiltration. (G): Aspect of the maxillary canine after infiltration. (H): Composite resin restoration of the hypoplasic area, using the infiltration resin as the bonding agent. (I): Restoration of the incisal defects. (J): Polishing of composite restorations. (K): Postoperative view, with satisfactory improvement of esthetic appearance. (L): Preoperative view of the right canine and lateral incisors. (M): Teeth after resin infiltration and restoration of canine. (N): Preoperative view of the central incisors. (O): Upper central incisors after infiltration of the labial side and restoration of the incisal border..

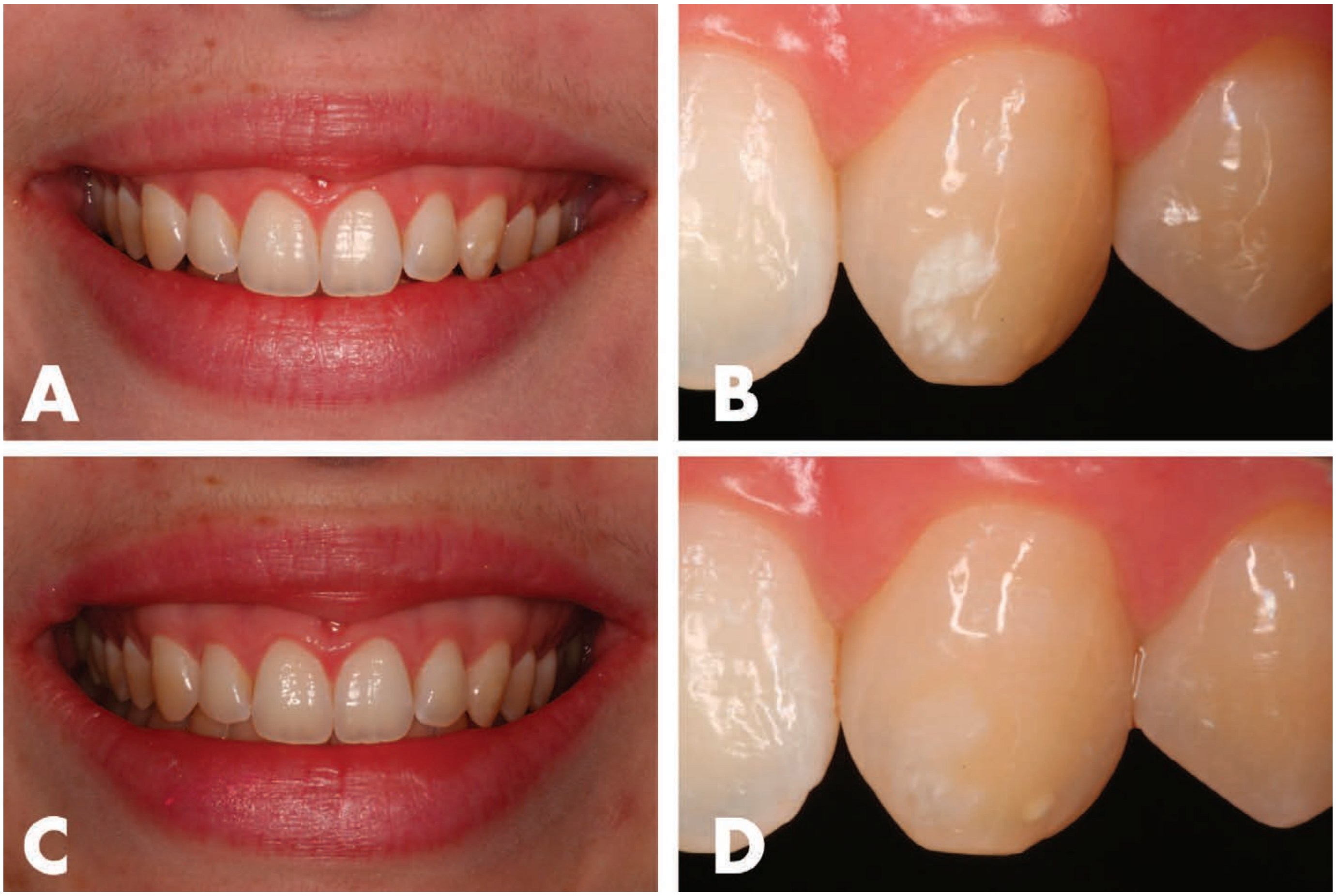


Figure 4. *Case 4, hypomineralized enamel defect, related to injury affecting the antecessor primary tooth. (A and B): Preoperative view, showing the white color alteration in the middle portion of the left superior canine, extending to incisal. (C and D): Postoperative view with improved esthetic appearance. Although some areas still exhibit staining, probably due to the greater depth, a satisfactory outcome was obtained.*

pressure, it invariably results in considerable enamel reduction.[21,22]

The resin infiltration technique is based on the penetration of a low-viscosity resin inside the capillary structure of the hypomineralized subsurface layer. Nevertheless, the surface layer hampers resin penetration because of its low pore volume. Thus, the hydrochloric acid is used to promote superficial erosion, removing about 30-40 μm of the surface, enabling the material to infiltrate the porous lesion.[23] Since the refractive index of the infiltrant material ($RI_{Icon} = 1.52$) is close to enamel ($RI_{hydroxyapatite} = 1.62\text{-}1.65$), when the lesion is filled, the optical properties of affected enamel are modified and lesions are masked.[17]

In the previously described white discolorations, the mineral phase is diminished and replaced by organic fluids, therefore altering the chemical composition of enamel. This causes a difference in the refractive index of enamel, since there are two different phases, altering the light scattering and causing the whitish appearance.[1]

Since the fluorotic and hypomineralized enamel exhibits a subsurface reduced mineral content, similar to an initial caries lesion, the indication of the low-viscosity infiltration resin technique was recently broadened to mask the undesirable esthetic appearance in these cases.[19,24] Nevertheless, because of the high variety of traumatic hypomineralization topographic characteristics, the results of treatment using infiltration are difficult to predict. In fact, this case series presents an improvement of the esthetic appearance of the white discolorations, with patient satisfaction. However, the masking effect was not always complete, mainly in the cases of traumatic hypomineralized discolorations. This may be related to the histology of these defects, since their depth and morphology are highly variable. In some cases, the defect presents a circular shape, forming an acute angle with the enamel surface,

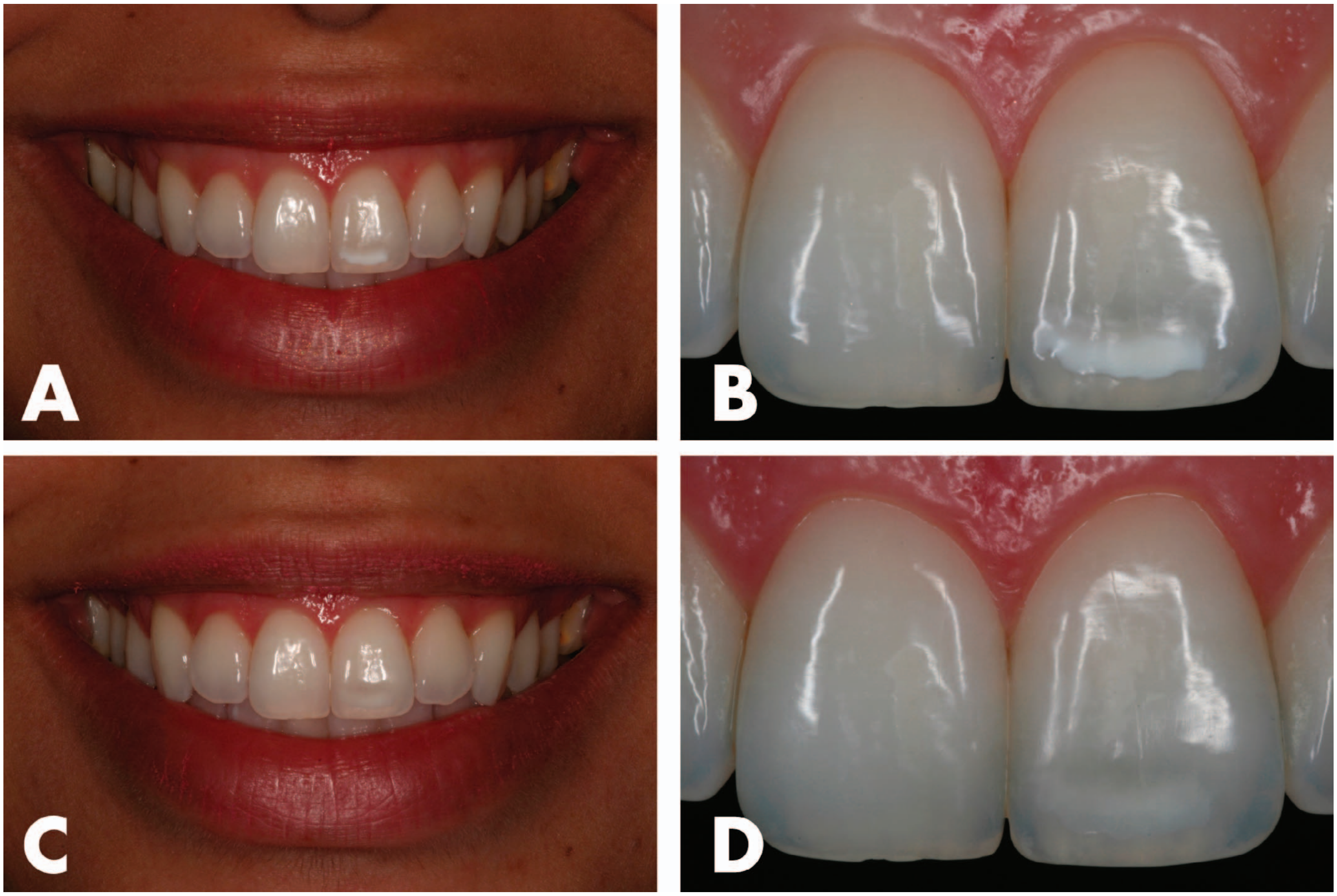


Figure 5. *Case 5, hypomineralized enamel developmental white defect, caused by trauma affecting the primary tooth. (A and B): Preoperative view, showing the delimitated white color alteration in the incisal part of the right central incisor. (C and D): Postoperative view with improved esthetic appearance. In this case, the hydrochloric acid was applied three times (two minutes each application). The lesion was satisfactory masked, although a slight white discoloration can still be observed after infiltration.*

which hampers the infiltration of the resin on the margins and results in a visual contouring of the lesion known as the "edge effect."[1] Since the hydrochloric acid eliminates the surface layer, it allows further infiltration into the center part of the lesion. If the margins of the lesion do not contact the enamel surface (because of the acute angle formed), the erosion does not occur in the surface of the lesion but in the surface of the sound enamel, preventing the penetration of the resin in the outline.[1] On the other hand, in fluorosis and carious white spot lesions, the angle formed between the lesion and the surface is obtuse, providing a more efficient infiltration of the lesion after erosion.[1]

A very important aspect related to the infiltration technique is the protection of the gingival margin, given that the hydrochloric acid should not contact the soft tissues. Especially in cases of fluorosis (and carious white spot lesions), the color alteration can be spread throughout the crown, reaching the cervical area of the tooth. Thus, the placement of the rubber dam is critical, since appropriate ligatures on all affected teeth are needed (as shown in cases 2 and 3). These ligatures retract the gingival tissue, exposing completely the cervical area of the tooth, allowing the infiltration of this region, and, consequently, avoiding the presence of white discolorations near the gingival margin, which would compromise the esthetic outcomes. However, this procedure should be carefully performed to avoid damage to the gingiva and reduce patient discomfort. The use of a liquid rubber dam is not appropriate in these cases, as it does not retract the gingival tissue and usually covers part of the cervical area of the tooth (as presented in case 1).

In cases in which an additional restorative intervention is combined with infiltration (when hypoplasic defects or cavities are associated with white spot areas), the infiltration resin can be used as bonding agent, since it is based on resinous monomers, facilitating the restoration technique and eliminating the need to use an adhesive system as a separate step.[25]

In addition, it should be pointed out that resin-based materials present an oxygen-inhibited superficial layer, which could be more susceptible to surface staining because of incomplete polymerization.[26] Attempts to deal with this are the application of an air barrier before light curing, such as glycerin gel, and/or surface polishing. The *in vitro* results of the staining behavior of the infiltration resin are not completely elucidated, but it was shown that it is important to polish the infiltrated lesions adequately to avoid further surface staining.[17] In a previous study, some staining was observed after immersion of infiltration-treated enamel in coffee and wine. Nevertheless, the repolishing of the specimens reduced these staining effects.[27]

CONCLUSIONS

It can be concluded that the resin infiltration technique can be successfully used to mask fluorosis and hypomineralyzed areas of enamel. This case series presented an improvement of the esthetic appearance of the lesions, with satisfactory outcomes, although in more severe cases, the color masking was not complete.

Conflict of Interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Clinical Research

Longitudinal Results of a 10-year Clinical Trial of Repair of Amalgam Restorations

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Clinical Relevance

Repair of amalgam restorations is a safe and effective treatment to increase the restorations' longevity with minimal intervention dentistry.

SUMMARY

The aim of this prospective, blind, and randomized clinical trial was to assess the effectiveness of repair of localized clinical defects in amalgam restorations that were initially scheduled for replacement. A cohort of 20 patients with 40 (Class I and Class II) amalgam restorations that presented one or more clinical features that deviated from the ideal (Bravo or Charlie) according to US Public Health Service criteria, were randomly assigned to either the repair or the replacement group—A: repair, n = 19; and B: replacement, n = 21. Two examiners who had calibration expertise evaluated the restorations at baseline and 10 years after according to seven parameters: marginal occlusal adaptation, anatomic form, surface roughness, marginal staining, contact, secondary caries, and luster. After 10 years, 30 restorations (75%) were evaluated (Group A: n = 17; Group B: n = 13). Repaired and replaced amalgam restorations showed similar survival outcomes regarding marginal defects and secondary caries in patients with low and medium caries risk, and most of the restorations were considered clin-

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ically acceptable after 10 years. Repair treatment increased the potential for tooth longevity, using a minimally interventional procedure. All restorations trend to downgrade over time.

INTRODUCTION

Amalgam has been the material of choice for stress-bearing dental restorations located in the posterior area. Despite the good long-term clinical results and relatively inexpensive cost,[1-3] this material does not fulfill the esthetic demand set by the public at large. Additionally, it does not provide adhesion to tooth structure.

Analyses of long-term restorations are an important factor for dentists, patients, and insurance companies. Amalgam presents limited longevity in the oral environment, which has been reported to be between 4.7 and 11.8 years of use.[4-6] Failures after this period have been associated with secondary caries, marginal deficiencies, degradation/wear, fracture, or loss of anatomic form.[5,7-16]

The replacement of failed restorations is the most common treatment in dentistry 10 years after initial placement, and long-term studies have shown that when failure takes place it typically happens within the first 24 months.[17]

Dentists frequently replace restorations that could be treated in a more conservative manner.[18,19] When a restoration is replaced, a significant amount of healthy tooth structure is removed and the preparation is subsequently enlarged. This negatively affects the longevity of the tooth.[20] Additionally, complete replacement of a restoration has the disadvantage of being more time consuming than a repair. There is also the added risk of converting the restoration to an indirect restoration or even the possibility of a major pulp-tissue injury.[5,21]

Repair rather than replacement of a failing restoration is part of minimally invasive dentistry, which seeks to ensure that healthy tooth structure is preserved. Early detection of carious lesions with minimal or no surgical interventions can lead to keeping teeth functional for life.[22,23]

Laboratory and clinical studies that compared replacement of defective restoration and restoration repair showed that repair was a simpler and less time-consuming procedure, thus improving the clinical properties of an otherwise defective amalgam restoration. The longevity of dental restorations increased considerably, and the repair was just as effective as a total restoration replacement, considering the minimal amount of intervention required and the lower cost involved. Repairing a restoration with a modified surgical approach that includes creating smaller tooth preparations and modifying the cavity-prep design could be, in many cases, the most conservative treatment option.[13,24-30]

Repair is an option for the treatment of defective amalgam restorations that present with localized defects. It involves the removal of the part of the restoration that is defective and any carious tissue adjacent and subjacent to the defective area and the remaining restoration of the prepared site.[25]

The aim of this prospective, blind, randomized cohort study was to assess the effectiveness of repairing localized clinical defects in amalgam restorations that were initially scheduled for restoration replacement.

The hypothesis was that the repair of amalgam restorations will improve their clinical conditions, increasing their longevity, similar to replacement of restorations.

METHODS AND MATERIALS

Study Design

A cohort of 20 patients between the ages of 18 and 80 years (mean, 26.5 years), women (58%) and men (42%) with 40 (Class I and Class II) amalgam restorations that presented one or more clinical features that deviated from the ideal (Bravo or Charlie, according to modified US Public Health Service [USPHS] criteria) were recruited at the Operative Dentistry Clinic of the University of Chile Dental School. The protocol was approved by the school's Institutional Research Ethics Committee (project PRI-ODO-0207), and all patients signed an informed consent form and completed a registration form. The protocol of the study was registered under No. NCT02051179 (ClinicalTrials.gov).

Inclusion Criteria—Those included were 1) patients having at least one tooth with a localized marginally defective amalgam restoration(s) that was clinically determined to be suitable for repair, according to USPHS criteria; 2) patients with at least 20 teeth; 3) restorations in functional occlusion with at least one opposing natural tooth; 4) patients who were asymptomatic of postoperative sensitivity; 5) patients with occlusal and proximal contact areas; 6) patients older than 18 years; 7) patients who agreed and signed the informed consent form for participating in the study, and 8) patients with areas outside the restoration's failure that were in good condition.

Table 1: *Modified USPHS Clinical Criteria*

Clinical Characteristic	Alpha	Bravo	Charlie
Marginal adaptation	Explorer does not catch or has one-way catch when drawn across the restoration/tooth interface	Explorer falls into crevice when drawn across the restoration/tooth interface	Dentin or base is exposed along the margin
Anatomic form	The general contour of the restorations follows the contour of the tooth	The general contour of the restoration does not follow the contour of the tooth	The restoration has an overhang
Surface roughness	The surface of the restoration does not have any surface defects	The surface of the restoration has minimal surface defects	The surface of the restoration has severe surface defects
Marginal staining	There is no discoloration between the restorations and tooth	There is discoloration on less than half of the circumferential margin	There is discoloration on more than half of the circumferential margin
Contact	Normal	Light	None
Secondary caries	There is no clinical diagnosis of caries	—	There is clinical diagnosis of caries
Luster of restoration	The restoration surface is shiny and has an enamel-like, translucent surface	The restoration surface is dull and somewhat opaque	The restoration surface is distinctly dull and opaque and is esthetically displeasing

Exclusion Criteria—Patients were excluded if they 1) had contraindications for regular dental treatment based on their medical history; 2) had special esthetic needs that could not be solved by repair treatments; 3) had xerostomia and/or were taking medication that significantly decreased salivary flow; 4) had a high risk for caries, or 5) had psychiatric or physical diseases that interfered with oral hygiene.

Treatment Group Criteria—Initially, 229 restorations (66 patients) were evaluated. Of those, 20 patients with 40 defective restorations were recruited and evaluated in accordance with the modified USPHS criteria. Restorations that were clinically diagnosed with secondary caries (Charlie) or undercontoured or overcontoured anatomic form defects and restorations with marginal defects (Bravo) were randomly assigned (Random Number Generator, Microsoft Excel 97) to either the repair or the replacement group. Diagnosis of active secondary caries was done according to Ekstrand's criteria.[31]

The groups were labeled A: repair, n=19 (Class I, n=8; Class II, n=11) and B: replacement, n=21 (Class I, n=9; Class II, n=12).

Restoration Assessment and Outcome Measurements

The quality of the restorations was scored according to the modified USPHS criteria (Table 1).[32] Two examiners underwent calibration exercises (J.M. and E.F., Cohen κ interexaminer coefficient 0.74 at baseline and 0.87 at 10 years). The blinded examiners of the treatment assessed the restorations independently by direct visual and tactile examination (mouth mirror, No. 5, Hu Friedy Mfg Co Inc, Chicago, IL, USA, and explorer, No. 23, Hu Friedy) and indirectly by radiographic examination (bitewing) at baseline (immediately after treatment) and 10 years after treatment. The seven examined parameters were marginal occlusal adaptation, anatomic form, surface roughness, marginal staining, occlusal contact, secondary caries, and luster (Table 1). If any difference was recorded between the two examiners and if they did not reach an agreement, a third clinician (G.M.), who also underwent calibration exercises, made the final decision.

A change from Bravo to Alpha was considered an improvement, and a change from Alpha to Bravo represented deterioration.

Treatment Groups

A: Repair—The clinicians (P.V. and C.M.) used carbide burs (330-010 Komet, Brasseler GmbH Co, Lemgo, Germany) to explore the defective margin, carious lesion, or anatomic form of the restorations. Part of the restorative material adjacent to the defect was removed as an exploratory procedure, thus allowing a proper evaluation and subsequent diagnosis of the extent of the defect. Provided that the defect was limited and localized, the clinician then removed any defective tooth tissue. Mechanical retention was used inside the existing amalgam restoration. Rubber dam isolation was used for this procedure. Repair of the restorations was carried out with a dispersed-phase amalgam (Original D, Wyckle Research Inc, Carson City, NV, USA).

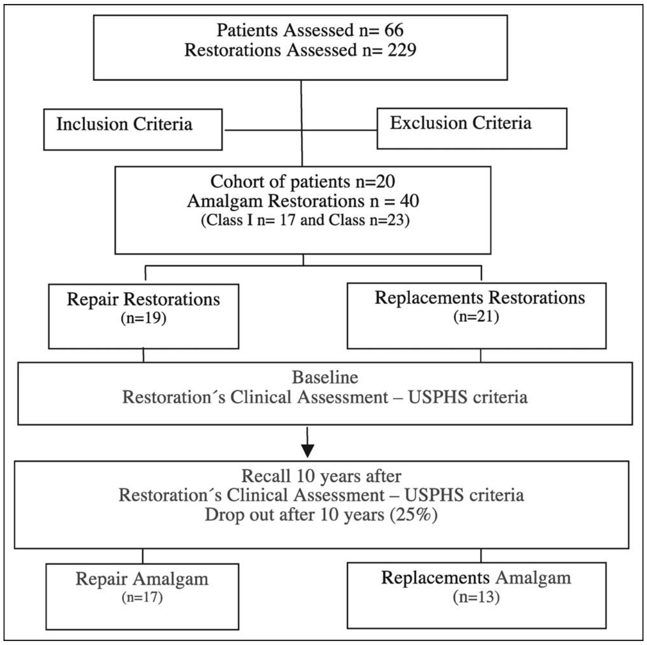

Figure 1. *Flow diagram of restorations, separated by group.*

B: Replacement—The clinicians completely removed and replaced the defective restorations. The removal of soft tooth tissue infected with caries was done using carbide high-speed burs under full water irrigation. After completing the cavity preparations, the tooth was restored with a new amalgam (Original D, Wyckle Research). Bonding agents and/or liners underneath the amalgam restorations were not used in this trial. Rubber dam isolation was used for all restorative treatments.

Patients were recalled four and 10 years after the restorations were placed for clinical assessment by the same examiners, who applied the same criteria used at baseline. Failed restorations were removed from the study and treated according to their diagnosed needs (Figure 1).

Statistical Analysis

The ordinal dependent variable was changed at the level of the modified USPHS criteria from the baseline value. The assigned score of each restoration reflected the worst result for any of the parameters. The results of each group in terms of degradation or upgrade were analyzed by the nonparametric Friedman range test to compare the preoperative and postoperative conditions. Additionally, the performance of all groups was contrasted using the Mann-Whitney test to determine the differences between the upgrade and downgrade of the restorations' quality. The statistical significance was set at 95%, α=0.05 and β=0.20; SPSS version 15.0 (SPSS Inc, Chicago, IL, USA) was used for statistical analysis.

RESULTS

Of the 40 restorations evaluated at baseline, 30 were returned for the 10-year recall (75%), group A: n = 17 and group B: n = 13. At the 10-year recall, two restorations were withdrawn from the study due to orthodontic reasons (metallic band covered the restorations), and patients who did not return for the recall were dropped (restorations withdrawn/dropped out = 2.5% per year).

In the marginal adaptation parameter, at baseline 31.58% of the repair group presented a Charlie value, as did 42.9% of the replacement group; neither group showed any Charlie after the initial intervention at the 10-year exam. Marginal adaptation at baseline presented 15.79% Alpha value for the repair group and 9.5% for the replacement group and after 10 years the Alpha value increased to 23.53% and 53.8%, respectively, (Figures 2a-3a).

The anatomic form presented at baseline was given a Charlie value in 26.3% of the repair group, and 9.5% in the replacement group; after treatment, no restorations were assessed as Charlie during the 10 years of observation. The Alpha value of the repair group increased from 15.8% at baseline to 23.5% after 10 years (Figures 2a, 3b), whereas the replacement group changed from 19.0% to 53.8% (Figure 3). All restorations from both groups were clinically acceptable after 10 years, according to the Friedman range test, which showed a parallel level of quality during the study period (Figures 2a, 2b and 3c).

The roughness parameter in the repair group began with an Alpha value of 47.4% at baseline, increased one year after intervention to 57.9%, and slowly decreased to 35.3% at the 10-year recall. The group's Charlie value was 0% at baseline and increased to 5.9% after 10 years; whereas, at baseline the replacement group showed an Alpha value of 28.6% and finished the study with 46.2% after 10 years. The Charlie value originally represented 19% and 0% of the replacement group at baseline and after 10 years, respectively (Figures 2a, 2b and 3d).

Marginal staining in both groups increased for the Alpha value after treatment, from 78.9% to 94.7% of repair and from 52.4 to 95.2% of replacement after the first year. Whereas repair showed a Charlie value at the third (5.9%) and fourth years (18.8%),

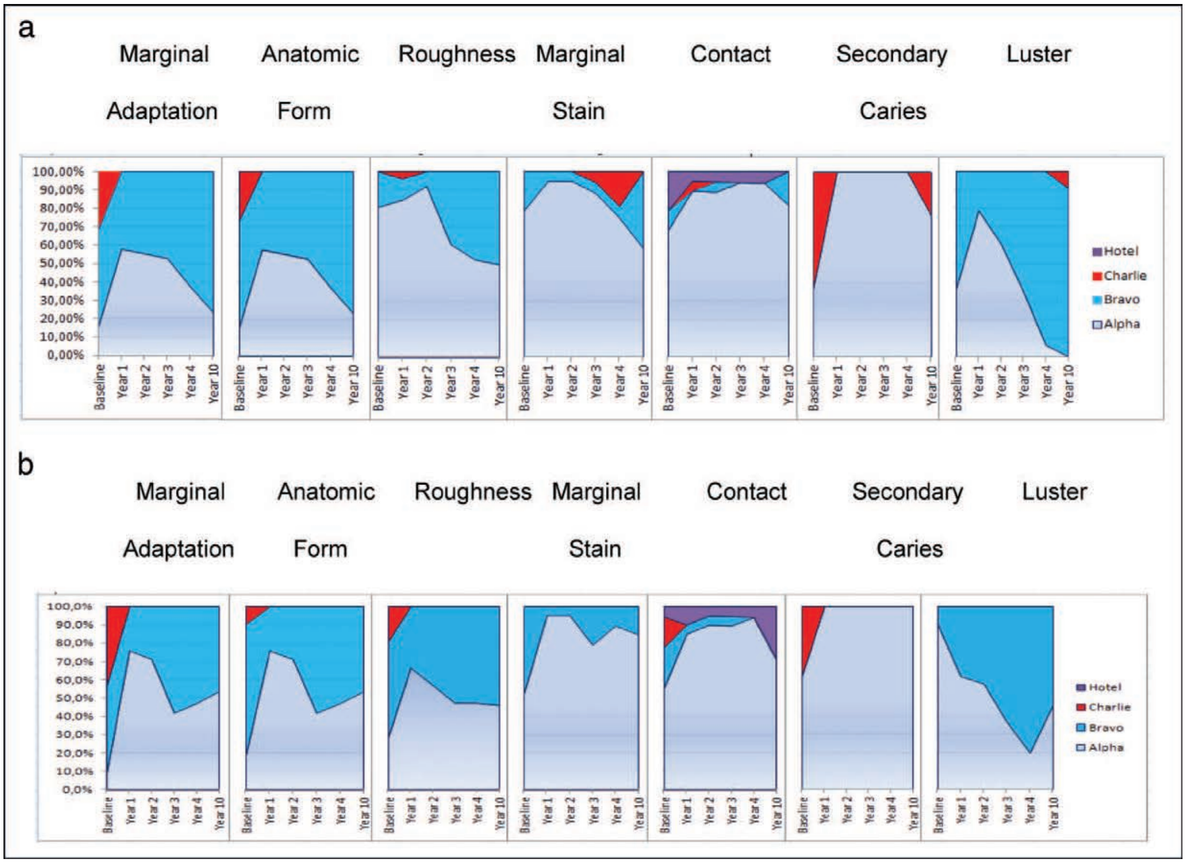

Figure 2. *(a) The area of the graph represents the different quality levels of the repaired restorations, expressed in percentages, separated by parameters and years of observation from baseline to 10 years. (b) The area of the graph represents the different quality level of the replacement restorations, expressed in percentages, separated by parameters and years of observation from baseline to 10 years.*

replacement did not present any Charlie values at the 10-year follow-up visit. In general, both groups showed a similar performance during all study periods, as per the Friedman test (Figures 2a, 2b and 3e). At the end of the study, 58.8% of the restorations in the repair group had an Alpha value compared with 84.6% of those in the replacement group.

The contact parameter of the repair group showed an Alpha value in 68.4% of the restorations and finished the study with 81.8%; whereas, the replacement group began with 55.6% of the restorations having an Alpha value and increased to 71.4% after 10 years. The overall value showed a reduction in the repair group from 21.1% at baseline to 0% at the end of the study; whereas, in the replacement group, it increased from 5.6% to 28.6% (Figures 2a, 2b, and 3f).

The secondary caries parameter showed a reduction in the repair group from 15.8% of Charlie value to 5.9% at the end of the study; whereas, the reduction in the replacement group was from 38.1% at baseline to 0% at the end. Neither group showed a Charlie case after the intervention and during the four years (Figures 2a, 2b, and 3g).

Finally, luster decreased in both groups: from 36.8% at baseline to 0% at the 10-year follow-up for the repair group and from 90.5% to 46.2% after 10 years for the replacement group. The Charlie value increased from 0% at baseline to 9.1% after 10 years for the repair group; whereas, the replacement group did not show any case of a Charlie value during the entire study period (Figures 2a, 2b, and 3g).

When comparing the restoration quality between the baseline and the 10-year follow-up, the groups showed statistically significant differences in the parameter anatomic form only, favoring the replacement group (Table 2).

DISCUSSION

Increased lifespan in humans to more than 80 years of age in numerous developed countries means that more individuals are reaching old age. From an oral-

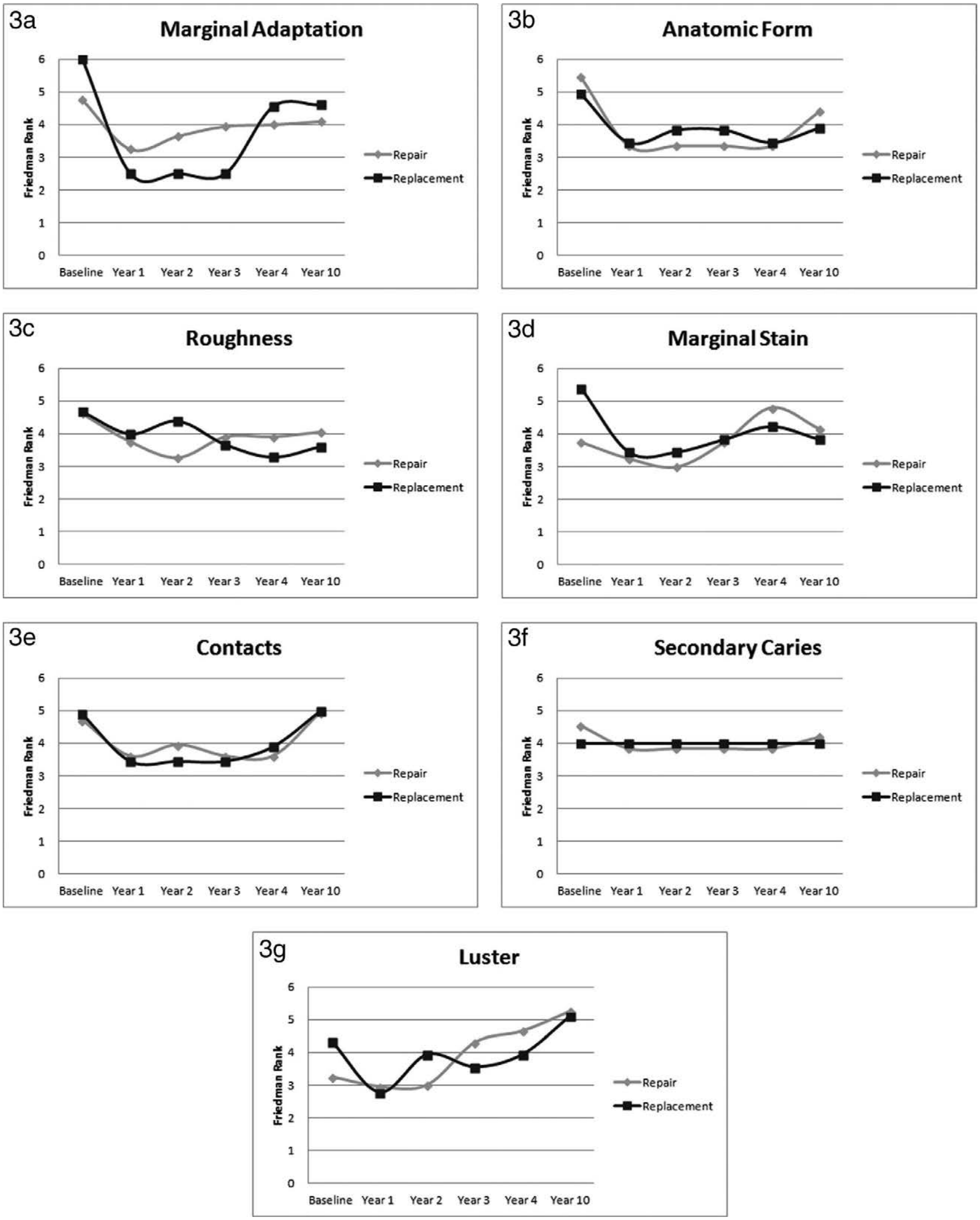

Figure 3. *(a) Marginal adaptation parameter, separated by group and year, indicates the distribution of the restoration quality, according to the Friedman Range Test. (b) Anatomic form, separated by group and year, indicates the distribution of the restoration quality, according to the Friedman Range Test. (c) Roughness, separated by group and year, indicates the distribution of the restoration quality, according to the Friedman Range Test. (d) Marginal stains, separated by group and year, indicates the distribution of the restoration quality, according to the Friedman Range Test. (e) The contact, separated by group and year, indicates the distribution of the restoration quality, according to the Friedman Range Test. (f) Secondary caries was separated by group and year. This figure indicates the distribution of the restoration quality, according to the Friedman Range Test. (g) Luster, separated by group and year, indicates the distribution of the restoration quality, according to the Friedman Range Test.*

health perspective, the increase in human longevity implies the need for prolonged tooth preservation.[33] Given the mechanisms and biological foundation of changes affecting teeth, it is necessary to apply novel dental therapies and adequate protective measures[34] to increase tooth longevity in the aging adult.

Moreover, dentistry has recognized the problem of the restorations' replacement, with more than 400 papers having been published during the past few years. The papers are related to the repair of restorations,[35] and many laboratory and clinical studies have shown that repairing amalgam restorations is possible and successful.[17,25,36-40]

This is the first prospective study that compared repair and replacement of localized restoration defects, and it was observed that both groups presented a similar level of quality after 10 years with the exception of the anatomic form parameter. This is easily explained, because repair included only a partial correction area of the restorations, and it is not possible to recover the total anatomy.

In general, restorations of both groups showed significant improvement during the first year after treatment in all parameters and began a slow and continued reduction of quality during all the study periods, as other research has shown.[4,17,25,41] Most of the restorations were assessed as clinically acceptable (Alpha or Bravo) after 10 years, and only a small percentage of restorations showed a Charlie value at the end of the study.

Parameters like marginal adaptation, roughness, and contact showed similar performance in both groups (no restoration with a Charlie score after 10 years), but marginal staining, secondary caries, and luster tend to result in more of a Charlie value in the repair group than the replacement group, although the differences were not statistically significant. Early downgrade was observed in the marginal staining parameter of the repair group (24.7% scored a Charlie during the third and fourth years), compared with no Charlie in the replacement group. The roughness of the repair group also scored a Charlie in the first year (5.3%), compared with no Charlie in the replacement group, but neither were statistically significant. This performance agrees with Gordan and others,[17] whom after seven years of amalgam repair observation noticed that there were no significant differences between study groups other than deterioration that had begun after the first two years.

In general, most of the Class I and Class II restorations in the repair group, downgraded to a Bravo after a period of three to four years, had a lower Charlie score at the end of the study in marginal adaptation, roughness, and luster. A revised analysis is necessary to determine whether it is possible to repair the restoration again and increase longevity of the restoration with a minimally invasive additional treatment to recover the Alpha score; however, the Bravo score did not represent a high risk of immediate restoration failure.[17]

For this study, limited and localized defects were considered as presence of secondary caries, undercontoured or overcontoured anatomic form, and marginal failures of occlusal, proximal, and cervical areas, clinically and radiographically detected. The accesses for repairing cervical and proximal defects included an occlusal approach in order to obtain complete visibility of the defect, given that the patients were mostly young adults; however, this practice could be different in the case of elderly patients, where probably, given gingival recession, the access to the cervical wall could be made by buccal or lingual faces or directly by the cervical area in some patients who had wide interproximal spaces.

The proposed hypothesis was confirmed: the Class I and Class II amalgam restorations showed similar results to the restorations that were repaired. By 10 years of observing the clinical characteristics, performance had increased with minimal intervention. Thus, the results provide evidence for clinicians to have a safe alternative to repair restorations that present with localized defects in marginal areas, including gaps with exposed dentin, loss of anatomic form, altered contact, or secondary caries. This observation will save time and healthy tooth tissue, lessen the need for anesthesia, and lower patients' stress as compared with the typical patient stress of traditional full restoration replacement Additionally, the results of this study support the idea that restoration repair could increase the longevity of restorations for least 10 additional years. The present study did not show any teeth or amalgam fracture or pulp injury during a 10-year period; those findings agree with previous reports that the strength of the repaired restoration was acceptable with no evidence of fracturing at the repaired interface.[42-44]

This increase in longevity and the fact that no additional treatment was required in the majority of the teeth studied for the repaired restoration group strengthens the hypothesis that restorations are able to function well without full replacement. However, the reasons for restoration downgrades

Table 2: *Comparison of the Seven Parameters, Between Baseline and 10 Years After, Between Groups by Mann-Whitney Test*

	Marginal Adaptation	**Anatomic Form**	**Roughness**	**Marginal Staining**	**Contact**	**Secondary Caries**	**Luster**
p value	0.52	0.001*	0.056	0.105	0.353	0.458	0.458

were not identified and the variables related to individual characteristics of the restorations were not evaluated (ie, flexion of the tooth cusp, problems related with deep carious lesions, size or design of the restoration, malocclusion, and bruxism). Those variables need to be further explored in order to get a representative statistical analysis for the downgrade scores.

Selecting criteria for making the decision to repair is one of most important problems to solve in treatment planning. It is necessary to clarify that a repair in the present study was indicated when the defects were localized and the other part of the restorations remained in acceptable clinical condition (Alpha or Bravo); it is considered to have been carried out with minimal intervention and implies the addition of new restorative material, with cavity preparation, in patients with standard oral health and low or medium caries risk. Like any other restorative treatment plan, the need for periodic monitoring, presence of marginal gaps with or without exposed dentin or ditching with or without marginal staining, diagnosed localized secondary caries, and wear of the restorations were the main indications of repair in the present study.[35] Most defects in restorations involve the cervical area; therefore, the early determination of the extension of the exploratory procedure is a key for the clinical decision to repair or to replace the restorations. It is not possible to indicate repair when secondary caries is not totally accessible with a minimally involved carious lesion or if it affects the restoration's resistance to typical functional forces, because deep involvement will undermine the carious lesion. Also, when restorations present with bulk defects they do not benefit from the alternative treatments. Those restorations are typically treated successfully if a complete replacement is done. Other contraindications must be considered when patients are reluctant to have a restoration repaired and would prefer to have a replacement due to prior history of a failed repair.[35]

New marginal carious lesions in teeth represent the greatest reason for failure. Caries represents an infection, which cannot be treated only by replacing or repairing the restoration. Practitioners must consider teaching the patient how to make changes in the biofilm with better oral hygiene practices. The restorative treatment is only a symptomatic treatment for secondary caries; the patient response, in addition to the quality of the restoration evaluation, must be considered with this factor.[45]

This alternative treatment represents an additional option to address many of the increasing number of restorations each year that require treatment due to deterioration. Repair instead of replacement of localized defective restorations significantly improved their morphology and function. The restorations showed some deterioration over time; however, they retained their acquired properties satisfactorily during the observation period in similar conditions other than replacement.[17,29,42,46] It is necessary to consider that repairing a restoration could serve to reduce the dimension of the repaired cavity, because it improves repair strength, and additionally it has been observed that rounded undercuts slightly reduce the repair strength values.[39]

A previous retrospective study by Smales and Hawthorne[47] compared the long-term survival rate of repaired vs replaced amalgam restorations, with no significant survival differences between both groups at year 5; however, higher failure rates of the repaired amalgam were seen after 10 years (only 37.2% of the restorations survived). In the present study, repair and replacement restorations did not show significant survival differences, when analyzing only the Alpha value, remained at an Alpha value for about five years, depending on the parameter, but later, restorations remained clinically acceptable because most of them retained a Bravo value at the end of the study period. Smales' study presented several differences with the current research; for example, the clinical criteria for repair indication and assessment criteria of the restoration was declared as a clinical judgment of the dentist, whereas the present study used calibrated clinicians and USPHS criteria. Additionally, the present study did not include data related to bulk amalgam fractures or cusp fractures. Another difference is that Smales' study[47] was carried out retrospectively in private practice and the present investigation was prospectively conducted in a university environment.

This study agreed with other studies when it suggested repairing restorations instead of replacement as it revealed broad clinical success when based on proper indications.[17,48]

This cohort includes data of restorations previously reported [25,27,29,30,46] and patients subsequently recruited. In a study by Fernandez et al.[49] data of resin composites restorations was reported with similar methodology. It is important to remark that, besides the difference in the material, the patient cohort was also different.

CONCLUSION

The present study supported the concept that a Class I or Class II amalgam restoration showed improvement and similar results when they were diagnosed with a localized marginal defect, a secondary carious lesion, wear, or an anatomic form defect and then treated by a repair or replacement restoration in patients with a low or medium caries risk, thus keeping the restorations clinically acceptable after 10 years. Repairing the restoration with minimal intervention dentistry procedures increased the longevity of the original restoration and the tooth itself. It is worth noting that all restorations trend to downgrade over time.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Laboratory Research

Transdentinal Cytotoxicity of Carbodiimide (EDC) and Glutaraldehyde on Odontoblast-like Cells

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Clinical Relevance

Carbodiimide (EDC) and glutaraldehyde are cross-linking agents capable of increasing resin-dentin bond durability. This study showed that these substances might be safely applied on acid-etched dentin since they have no transdentinal cytotoxic effects on cultured odontoblast-like cells.

SUMMARY

Objective: To evaluate the transdentinal cytotoxicity of three different concentrations of carbodiimide (EDC) or 5% glutaraldehyde (GA) on MDPC-23 cells.

Methods: Seventy 0.4-mm-thick dentin disks obtained from human molars were adapted to artificial pulp chambers. MDPC-23 cells were seeded on the pulpal surface of the disks. After 48 hours, the occlusal dentin was acid-etched and treated for 60 seconds with one of the

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following solutions (n=10): no treatment (negative control); 0.1 M, 0.3 M, or 0.5 M EDC; 5% GA; Sorensen buffer; or 29% hydrogen peroxide (positive control). Cell viability and morphology were assessed by methyltetrazolium assay and scanning electron microscopy (SEM), respectively. The eluates were collected after the treatments and applied on MDPC-23 seeded in a 24-well plate to analyze cell death, total protein (TP), and collagen production. The last two tests were performed 24 hours and seven days after the challenge. Data were analyzed by Kruskal-Wallis and Mann-Whitney tests ($p<0.05$).

Results: EDC at all test concentrations did not reduce cell viability, while 5% GA did increase cell metabolism. Cell death by necrosis was not elicited by EDC or 5% GA. At the 24-hour period, 0.3 M and 0.5 M EDC reduced TP production by 18% and 36.8%, respectively. At seven days, increased TP production was observed in all groups. Collagen production at the 24-hour period was reduced when 0.5 M EDC was used. After seven days, no difference was observed among the groups. SEM showed no alteration in cell morphology or number, except in the hydrogen peroxide group.

Conclusions: Treatment of acid-etched dentin with EDC or GA did not cause transdentinal cytotoxic effects on odontoblast-like cells.

INTRODUCTION

The biodegradation of resin-dentin bonds is a complex process that involves the leaching of unpolymerized monomers that infiltrated the demineralized dentin matrix and the enzymatic cleavage of exposed collagen fibrils.[1,2] Therefore, the resistance of the adhesive interface components against degradation determines its stability and durability.[1,3-5]

Collagen fibrils that are not encased by monomers during the bonding procedure,[6,7] as well as those exposed by the polymer degradation over time, are highly susceptible to enzymatic hydrolysis.[8-12] Matrix metalloproteinases (MMPs) and cysteine cathepsins have been identified in sound and caries affected-dentin[8,12-15] and are allegedly responsible for the disappearance of portions of the hybrid layers.[11,16,17] Thus, improving collagen's resistance against enzymatic degradation and inactivating these proteases are important steps to enhance the quality and longevity of dentin bonding.

The treatment of demineralized dentin with EDC (1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride) or glutaraldehyde (GA) increases the mechanical properties of dentin collagen[18] and inhibits MMPs[19,20] by creating new cross-links among protein peptide chains. EDC is capable of forming covalent peptide bonds between peptide chains by activating the free carboxyl group of glutamic and aspartic acids,[21,22] while GA reacts with the ε-amino groups of lysyl or hydroxylysyl residues to induce the formation of intra- and intermolecular cross-links.[23,24]

Although the effects of EDC and GA have been demonstrated on collagen biomodification,[18,25] rendering collagen more resistant to degradation by improving its mechanical properties, such as elastic modulus and ultimate tensile strength, and MMP inhibiton,[19,20] there is little information available about their cytotoxicity. The carbodiimides have shown better results than has GA in terms of the biocompatibility on U937 macrophage-like cells,[26] rat ocular cells/tissue,[27] and corneal endothelial cells.[28] However, there are no studies that have tested the transdentinal cytotoxicity of these cross-linking agents on odontoblast-like cells beneath dentin to determine and confirm if these substances are safe when applied on acid-etched dentin.

Thus, the aim of this study was to evaluate the transdentinal cytotoxicity of different concentrations of EDC or 5% GA on odontoblast-like cells. The tested null hypothesis was that the treatment of acid-etched dentin with cross-linkers for 60 seconds does not exert cytotoxic effects on the target cells.

METHODS AND MATERIALS

Preparation of Dentin Disks and Permeability Reading

Seventy sound human third molars were obtained after approval was received by the Ethics Committee of the School of Dentistry at Araraquara–UNESP; these teeth were stored in 0.12% thymol solution at 4°C. The teeth were used within three months after extraction. One 0.5-mm-thick dentin disk was obtained from the mid-coronal dentin of each tooth using a precision cutting machine equipped with a water-cooled diamond saw (Isomet 1000, Buehler Ltd, Lake Bluff, IL, USA). The disks were carefully examined with a stereoscopic microscope (SZX7, Olympus, São Paulo, SP, Brazil) to confirm the absence of enamel islets and defects resulting from pulp horn projections. Then the occlusal side of the disks was manually finished with wet 320-grit silicon carbide paper to reach a final thickness of 0.4 mm, as measured with a digital caliper accurate to the nearest 0.01 mm (Mitutoyo South Americana Ltd, Suzano, SP, Brazil).

Dentin permeability was determined to permit a homogeneous distribution of the dentin disks into the groups. The smear layer produced on both sides of the disks was removed by application of 0.5 M ethylenediamine tetraacetic acid (pH 7.4) for 60 seconds, followed by abundant rinsing with deionized water. For determination of the hydraulic conductance, the disks were individually placed in *in vitro* pulp chambers (IVPCs) modified from Hanks and others.[29] A metallic cannula connected the IVPC to a 180-cm column of water. The disk remained under this pressure for five minutes, after which time the movement of a microbubble introduced through the cannula was recorded over the course of one minute, and the obtained values were transformed into conductance values. Then the dentin disks were allocated into seven groups (n=10) in such a way that the dentin permeability was statistically similar among the groups (analysis of variance, $p>0.05$).

MDPC-23 Cell Seeding on Dentin Disks

MDPC-23 cells were cultivated in Dulbecco Modified Eagle Medium (DMEM; Sigma Aldrich Corp, St Louis, MO, USA) containing 10% fetal bovine serum (Cultilab, Campinas, SP, Brazil), 100 IU/mL penicillin, 100 μg/mL streptomycin, and 2 mmol/L glutamine (Gibco, Grand Island, NY, USA) in a humidified incubator with 5% CO_2 and 95% air at 37°C (Isotemp Fisher Scientific, Pittsburgh, PA, USA). The cells were subcultured each three days until the number of cells necessary to perform the study was reached.

To simulate clinical conditions, the smear layer was recreated on the occlusal side of each disk with a 600-grit silicon carbide paper for 10 seconds. Then the disks were placed in modified IVPCs and were both sterilized in ethylene oxide. MDPC-23 cells (3×10^4) were seeded on the pulpal side of the dentin disks (0.28 cm^2). In order to accomplish that, the IVPCs were placed in an inverted position (pulp side up) into the compartments of 24-well plates (COSTAR 3595, Corning Incorporated, Corning, NY, USA) and maintained in an incubator with 5% CO_2 and 95% air at 37°C for 48 hours to ensure adherence of cells. After this time, the IVPCs were carefully removed from the compartment and returned to the same compartment with the occlusal side up to receive the treatment solutions.

Application of the Cross-linking Solutions

Four treatment solutions (10 disks per solution) were prepared by diluting the cross-linking agent in Sorensen buffer (pH 6.2): 0.1 M EDC (pH 6.04); 0.3 M EDC (pH 5.98); 0.5 M EDC (pH 5.93), and 5% GA (pH 5.8). In addition, three more conditions were tested (n=10): Sorensen buffer only, 29% hydrogen peroxide (positive control), and no treatment (negative control). The EDC and GA concentrations were chosen based on satisfactory results observed in previous studies regarding MMP inhibition and increase in collagen mechanical properties.[19,20,30,31]

The occlusal surface of the dentin disks was etched with 35% phosphoric acid (Scotchbond etchant, 3M ESPE, St Paul, MN, USA) for 15 seconds, carefully rinsed with deionized water for 10 seconds, and blot-dried with sterilized cotton pellets. Then 20 μL of the predetermined treatment solution (cross-linking solutions, Sorensen buffer, or controls) was applied for 60 seconds[19,20,31]; this step was followed by water rinsing and blot-drying. All procedures were performed in a vertical laminar flow chamber to prevent contamination, and immediately afterward, the IVPCs were placed again in a CO_2 incubator for an additional 24 hours.

Analysis of Cell Viability (Methyltetrazolium [MTT] Assay)

Eight out of the 10 disks of each group were randomly selected for cell viability analysis using the MTT assay. The eluates (DMEM + products that diffused through the dentin disks) from each well were collected and frozen for subsequent analysis of type of cell death as well as type 1 collagen and total protein production. After 24-hour incubation, the disks were removed from the IVPCs and individually placed in the sterilized wells of new 24-well plates with the pulpal side containing the MDPC-23 cells turned upward. Then 900 μL of fresh DMEM and 100 μL of MTT solution (Sigma-Aldrich) (5 mg/mL in sterile phosphate-buffered saline [PBS]) were placed in contact with the disks. The cells were incubated with the MTT solution at 37°C for four hours. Next, the MTT solution was aspirated and 400 μL of acidified isopropanol solution was added (0.04 N HCl) in each well to dissolve the violet formazan crystals, producing a homogeneous purple solution.

Three 100-μL aliquots of each well were transferred to wells of 96-well plates (Costar Corp, Cambridge, MA, USA) and read at 570-nm wavelengths with an enzyme-linked immunosorbent assay (ELISA) plate reader (Thermo Plate, Nanshan

District, Shenzhen, Gandong, China). The values obtained from the three aliquots were averaged to provide a single value for each sample.

Scanning Electron Microscopy (SEM) Cellular Morphology Analysis

Two disks from each group were randomly selected and prepared for SEM analysis. The cells were fixed in 2.5% GA (Sigma-Aldrich) in PBS for one hour at room temperature. Next, the glutaraldehyde was aspirated and the cells were rinsed with PBS, postfixed with 1% osmium tetroxide (Electron Microscopy Science, Fort Washington, PA, USA) for one hour, and rinsed again with PBS, followed by dehydration with ascending series of water–ethanol solutions (30%, 50%, 70%, 95%, and 100%) two times for 60 minutes each.

The cells were immersed for 60 minutes (three 20-minute changes) in 1,1,1,3,3,3-hexamethyldisilazane (ACROS Organics, Morris Plains, NY, USA). Finally, the specimens were mounted on metallic stubs and stored in a desiccator for 24 hours and sputter-coated with a gold layer (SDC 050; Bal-Tec AG, Balzers, Germany), and their morphology was examined with SEM (DSM 960, Carl Zeiss Inc, Oberköchen, Germany).

Cell Membrane Damage Measurements (Cell Death)

To analyze the cell membrane damage the Live/Dead Cell Viability/Cytotoxicity Kit (Invitrogen, San Francisco, CA, USA) was used. This assay uses the fluorescence probe ethidium homodimer-1 (EthD-1) that binds to DNA bands only in cells with cell membrane rupture. The second probe was the Calcein AM (CA), which is hydrolyzed by cytoplasmic esterases in viable cells. MDPC-23 cells were seeded in a 24-well plate (n=5) and exposed to the eluate collected after the dentin treatment for 24 hours. Then the supernatant was centrifuged (4000 rpm for two minutes), resuspended with DMEM, and returned to its original well. The plate was centrifuged (4000 rpm for two minutes) in order to allow the cells to precipitate, and the cells were incubated with 2 mM CA and 4 mM Eth-1 and Hoechst (1:5000) for nuclear staining, over the course of 15 minutes. The fluorescence was analyzed by an In Cell Analyzer 2000 (GE Healthcare Life Sciences, Freiburg, Germany) in six fields per well. The percentage of dead (positive Eth-1 staining) and live (positive CA staining) cells were calculated from the cells stained with Hoechst with the software In Cell Investigation (GE Healthcare Life Sciences), and the average value per well was used for statistical analysis.

Total Protein (TP) Production

Total protein (TP) production was evaluated according to the Read and Northcote protocol (1981), as previously described by Basso and others.[32] The eluate collected after dentin treatment (n=5) was placed in contact with MDPC-23 cells seeded in a 24-well plate for 24 hours. TP production was also evaluated seven days after placement in contact with EDC solutions. In both instances, the eluates were frozen for total collagen production detection and the cells were washed three times with 1 mL PBS at 37°C, and 1 mL of 0.1% sodium lauryl sulfate (Sigma-Aldrich Corp) was added to each well for 40 minutes at room temperature to produce cell lysis. After homogenization, an aliquot of 1 mL of each well was transferred to Falcon tubes, while the blank tube received 1 mL of distilled water (TP protocol was performed in 24-well plates, not in Falcon tubes). Next, the Lowry reagent solution (Sigma-Aldrich Corp) was added (1 mL) to all samples, the tubes were agitated for 10 seconds, and after 20 minutes, 500 mL of Folin-Ciocalteau's phenol reagent solution (Sigma-Aldrich Corp) was added to each sample and homogenized. Three 100-μL aliquots of each tube were transferred to a 96-well plate after 30 minutes, and the absorbance was read at 655 nm in an ELISA plate reader (Thermo Plate). The average of the three values was used for statistical analysis. Absorbance values were transformed into percentage, and the average of the control group was considered 100% of TP production.

Collagen Production

Collagen production by MDPC-23 cells was evaluated 24 hours and seven days after contact with EDC or GA solutions. The 24-hour and seven-day eluates were thawed and a 200-μL aliquot from each tube was transferred to a sterilized well of new 24-well plates. Then 500 μL of 0.1% Sirius Red dye (Sirius Red powder in picric acid, Sigma-Aldrich) was added to each well and the plate was incubated with shaking for one hour at 37°C. The content of each well was placed in 1.5-mL tubes, thus enabling sample centrifugation at 12,000 rpm. Supernatant was discarded and the precipitate was rinsed with 750 μL of 0.01 M HCl to each tube. Once again, samples were centrifuged for 10 minutes, the supernatant was discarded, and 250 μL of 0.5 M NaOH was added to solubilize the precipitate. After that, three 100-μL aliquots were transferred to a 96-well plate and read at 555 nm in a 96-well plate reader (Synergy H1 Hybrid Reader, Biotek, Winooski, VT, USA). The values obtained from the three aliquots were averaged to provide a single value for each sample. Total production of collagen was calculated based on a standard curve performed with predetermined concentrations of this protein.

Statistical Analysis

Data from the response variables (cell viability, cell death by necrosis, production of TP, and type I collagen) were not normally distributed and were analyzed by the application of Kruskal-Wallis and Mann-Whitney nonparametric tests. The latter was used for pairwise comparison between groups. For all statistical tests, $p < 0.05$ was taken to be statistically significant.

RESULTS

MDPC-23 cell viability and death by necrosis after contact with the transdentinal diffused components of different concentrations of EDC or 5% GA are shown in Figure 1. None of the EDC concentrations differed from the negative control or Sorensen buffer with regard to cell viability (Figure 1a). Only 5% GA increased the cell viability, although it was not statistically different from the EDC solutions, regardless of their concentrations. Cell death by necrosis (Figure 1b) was not increased by any of the investigated EDC concentrations or by 5% GA. The percentage of cell death varied from 0.5% to 1.9% for EDC groups. Variations in TP production are expressed as a percentage in Table 1; all of the values were calculated based on 24-hour–control. At 24 hours, only 0.3 M and 0.5 M EDC negatively interfered with TP production. Compared to the control (101.2%), the cell viability for these groups was 82.0% and 63.2%, respectively (Table 1, column). Lack of effect on TP production was seen for 0.1 M EDC and 5% GA, which did not differ from the control. However, at seven days, a significant increase in TP production occurred in all groups, compared to the 24-hour period (Table 1, rows). Collagen production at the 24-hour period was significantly reduced only by 0.5 M EDC in comparison to the negative control. Significant increase in the production of collagen from 24 hours to seven days was seen only for 0.5 M EDC (Table 2). The SEM analyses confirmed the results obtained with the MTT and Live/Dead assays. A large number of odontoblast-like MDPC-23 cells remained adhered to the dentin disks in all groups (Figure 2a-g), except in the positive control (29% hydrogen peroxide, Figure 2h). In the latter, wide cell-free zones and large areas of residual fragments of dead cells were observed. In the negative control, EDC, and GA groups, the cultured cells were near to confluence and exhibited abundant cytoplasm with numerous thin cytoplasmic projections that seemed to be adhering the cells to the dentin substrate (Figure 2b).

DISCUSSION

This study evaluated the transdentinal cytotoxicity of EDC solutions on odontoblast-like cells. The IVPCs containing dentin disks were used in order to simulate extremely deep cavities in which MDPC-23 cells were indirectly exposed to the tested solutions. This *in vitro* test simulates the *in vivo* condition in which the odontoblasts are the first cells to be in contact with components that have diffused through the dentin.[33] The sound dentin is a tubular mineralized matrix capable of protecting the pulp, even at a thickness as thin as 0.5 mm.[29,33] To increase the probability of the cytotoxic potential of each solution, 0.4-mm-thick disks were used in the present study.

EDC has been investigated in dentistry as a cross-linking agent that when applied directly to demineralized collagen increases its resistance against enzymatic degradation[18] and inhibits the endogenous MMPs of dentin.[19,20,34] Additionally, EDC is able to preserve the resin-dentin bond over time.[35] In the medical field, EDC has been used to reinforce different structures for tissue engineering applications and has shown satisfactory biocompatibility. This agent may support drug delivery system development,[36] bioprosthetic heart valve construction,[26] preparation of collagen scaffolds,[37] and a large number of other purposes.[38-41] However, these procedures use EDC to treat biomaterial structures prior to their contact with cells/tissues. The same is not observed when EDC is applied on dentin, since its fluid-filled tubular and porous structure allows EDC to diffuse across dentin to reach the odontoblast-like cells following its application. However, dentin thickness is known to dissipate the concentration of potential toxins over distance.[42]

GA is used in many different processes as a fixative, cross-linking, and disinfecting agent. As is the case for EDC, GA participates in the construction of bioprosthetic heart valves,[26,43] inhibits proteases,[31] and modifies gelatins and other materials and tissues.[26,44,45] In dental treatments, GA has been used as a desensitizer that reacts with plasma proteins in dentin fluid to precipitate them, blocking

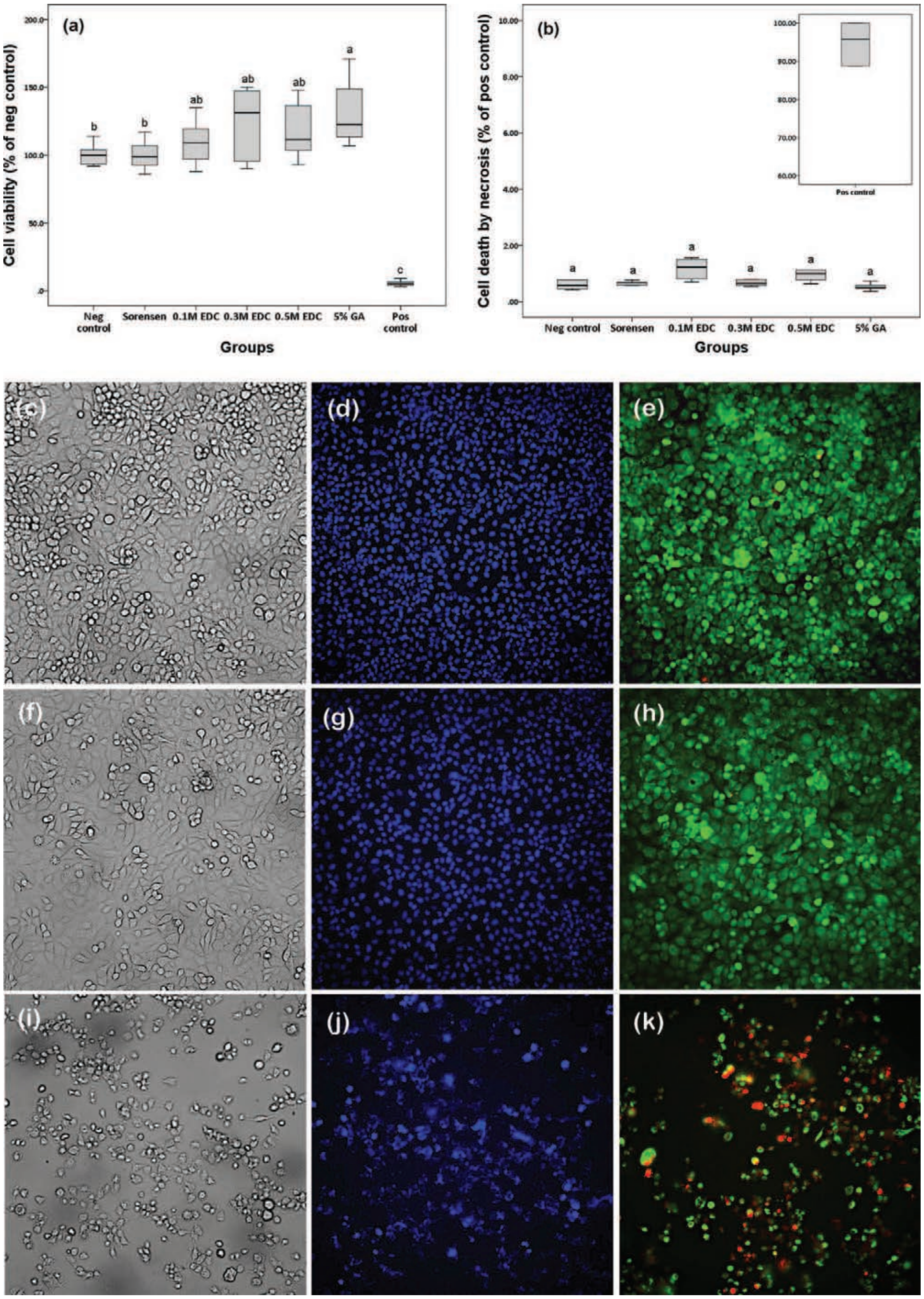

Figure 1. *Response of MDPC-23 cells after transdentinal contact with different concentrations of EDC. (a) Percentage of cell viability (MTT assay) related to the negative control (=100% of viable cells). (b) Percentage of necrotic dead cells related to the positive control (=100% of cell death). Groups identified by the same letter do not differ statistically (Mann-Whitney,* p>*0.05). (c-k) Representative confocal images of MDPC-23 cells co-stained with LIVE/DEAD® assay Kit after contact with Sorensen buffer (c, d, and e), 0.5 M EDC (f, g, and h), and positive control (i, j, and k). Live cells fluoresce green on Calcein dye uptake, and necrotic cells fluoresce red on ethil homodimer-1 uptake. Hoechst dye (blue) shows cell nucleus, representing total cell number observed in the white field. Magnification 20×.*

dentin tubules.[46-48] Wiegand and others[49] showed that a GA-containing dentin desensitizer applied on 1.0-mm-thick dentin specimens under simulated pulpal flow conditions did not present toxic effects on L-929 fibroblasts. The same was observed by Camps and others[50] using 0.5-mm-thick dentin slices. In agreement with those authors, the results of this study showed that 5% GA did not exert harmful effects on MDPC-23 cells when applied on 0.4-mm-thick dentin disks.

Table 1: *Production of Total Protein (% of Negative Control at 24 hours) by MDPC-23 Cells After Transdentinal Contact with Different Concentrations of Carbodiimide (EDC)*[a]

Groups	Period of Evaluation		
	24 h		7 d
Negative control	101.2 (100.7-101.2) A	*	250.4 (241.0-254.2) BC
Sorensen	108.8 (105.8-111.9) A	*	262.4 (254.8-263.6) AB
0.1 M EDC	95.1 (81.5-97.7) AB	*	244.1 (243.5-245.4) C
0.3 M EDC	82.0 (82.0-84.0) BC	*	263.6 (261.7-267.4) AB
0.5 M EDC	63.2 (59.7-64.7) C	*	256.1 (248.5-262.4) BC
5% GA	87.0 (78.9-87.0) AB	*	271.8 (263.6-272.4) A

Abbreviation: GA, glutaraldehyde.

[a] *Numbers are median (percentile 25-percentile 75), $n=5$. Within each column, groups followed by the same letter are not statistically different (Mann-Whitney, $p>0.05$).*

* *Indicates statistically significant difference between periods of evaluation (Mann-Whitney, $p>0.05$).*

In the present study, the cross-linking solutions were applied for one minute on the occlusal side of acid-etched dentin disks, which were then rinsed with deionized water prior to the analysis of the solutions' cytotoxic effects. The MTT assay revealed that none of the EDC concentrations differed from the negative control or Sorensen buffer with regard to cell viability. While 5% GA was able to slightly increase the cellular metabolism, the good results may have been favored by the water rinsing that removed the residual reagents after the treatment.

Cell death by necrosis occurs after irreversible cell damage generated by a chemical, physical, or biological injury. The Live/Dead assay data showed that EDC and GA presented no difference with regard to the percentage of cell necrosis compared to the negative control group (no treatment). In contrast, 29% hydrogen peroxide (positive control) induced between 90% and 100% cell death by necrosis. Corroborating cell viability and cell death findings, SEM observations, that were used in this study as a descriptive and qualitative method, showed no significant alterations on the phenotypical characteristics and/or number of cells to all groups, except for H_2O_2, the positive control.

The TP and collagen production were analyzed 24 hours and seven days after the application of the treatment solutions. Only 0.3 M and 0.5 M EDC significantly reduced 24-hour TP production compared to the control group. However, this reduction was no longer significant after seven days, which suggests that if EDC caused any alteration in the cell function it was reversible in a short period of time and did not affect cell viability. Treatment with 5% GA had been similar to the control group (no treatment) at 24 hours; it was not observed after seven days when GA, 0.3 M EDC, and Sorensen buffer treatments increased TP production. The analysis of collagen concentration in the eluate showed that 0.5 M EDC reduced collagen production 24 hours after its application. However, after seven days, no difference was observed in any treated group when compared to the control.

The results from the different tests are in agreement, showing that EDC or GA applied on acid-etched dentin for 60 seconds and then rinsed

Table 2: *Type I Collagen Production (% of Negative Control at 24 hours) by MDPC-23 Cells Exposed to Cell Culture Medium Beneath Acid-etched Dentin Treated with Carbodiimide (EDC) or Glutaraldehyde (GA)*[a]

Groups	Period of Evaluation		
	24 h		7 d
Negative control	100.4 (97.1-103.0) A	n.s.	100.3 (96.3-103.2) AB
Sorensen	102.0 (97.1-104.8) A	n.s.	94.9 (91.0-97.4) B
0.1 M EDC	97.7 (91.1-104.7) AB	n.s.	89.7 (80.0-97.5) B
0.3 M EDC	106.7 (103.1-110.7) A	n.s.	92.9 (92.4-93.3) B
0.5 M EDC	83.8 (74.2-91.4) B	*	98.1 (96.3-100.8) B
5% GA	94.2 (87.2-102.6) AB	n.s.	105.3 (104.2-106.5) A

[a] *Numbers are median (percentile 25-percentile 75), $n=5$. Within each column, groups followed by the same letter are not statistically different (Mann-Whitney, $p>0.05$).*

* *Indicates statistically significant difference between periods of evaluation, while n.s. (not significant) indicates absence of significance (Mann-Whitney, $p>0.05$).*

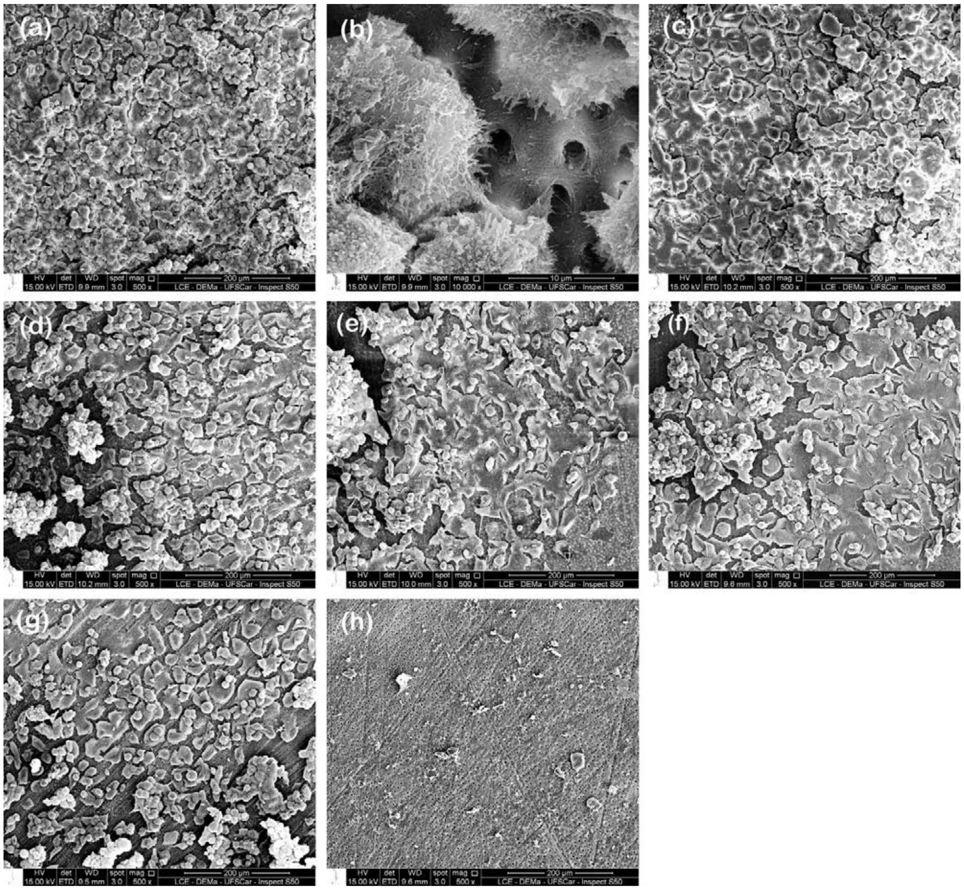

Figure 2. *Composite figure of SEM micrographs representative of negative control (a and b), Sorensen buffer (c), 0.1 M EDC (d), 0.3 M EDC (e), 0.5 M EDC (f), 5% glutaraldehyde (g), and 29% hydrogen peroxide (positive control) (h). SEM 500×. A large number of MDPC-23 cells remained attached to the dentin surface, except in the positive control group (h). In that particular group most cells detached from the dentin, and only residual fragments of dead cell are seen. In (b) it is possible to observe the large number of microextensions of the cells, responsible for the cell fixation to the dentin substrate seen in the background of the image. SEM 10,000×.*

with deionized water did not exert cytotoxic effects on odontoblast-like cell MDPC-23 even when the remaining dentin was as thin as 0.4 mm. These results require acceptance of the tested null hypothesis. In addition, MTT data associated with TP production seem to indicate that GA was able to slightly increase cell metabolism, the opposite of what was expected.

Some critics may wonder why EDC and GA were not cytotoxic when applied topically to dentin, while they have been shown to be cytotoxic when applied directly to cells. The answer lies in the structure and function of dentin as a protective barrier. Dentin protects the pulp from toxic chemicals in at least three ways: 1) the surface area of fluid-filled dentinal tubules only represents 1% of the physical surface area. That is, mineralized matrices occupy 99% of the surface area of superficial dentin. In deep dentin, open dentinal tubules may occupy up to 22% of the area, while mineralized matrix occupy 78% of the physical surface area.[51] Thus, based on diffusional surface area, dentin restricts diffusion. 2) As solutes diffuse down dentinal tubules, they often bind to hydroxyapatite lining the tubules or the collagen fibrils within the lumen. GA has been shown to react with albumin and other plasma proteins present in dentinal fluid to form insoluble precipitates of proteins that tend to decrease dentin permeability.[47,48] 3) The chemical concentration driving diffusivity is the applied concentration that dissipates over distance. That is, the applied concentration rapidly falls the further the solute diffuses into dentin, so that by the time it reaches the other side of the dentin, its concentration has fallen by a 10-, 100-, or 1000-fold measure.[42] Finally, when the dentin surface is rinsed with water to stop inward diffusion by dilution, the solutes in dentin start to diffuse backwards out of the tubules, since there is more solute in the tubules than at the water rinsed surface. All of these processes protect the pulpal cells from solutes applied to dentin surfaces. If one by-passes the protective effect of dentin by, for instance, applying EDC directly to pulpal soft tissue exposed by a pulp exposure, then pulpal cells would be exposed to much higher EDC concentrations (by 10^3-10^5) than occur following application to intact dentin. This pioneering study in transdentinal cytotoxicity of cross-linking agents showed that the use of these substances in dental practice to enhance resin-dentin bond quality may be safe. Future studies are necessary to better understand the interaction of these agents with pulp tissue and their behavior *in vivo*.

CONCLUSIONS

According to the methodology used in the present investigation, it may be concluded that the treatment of acid-etched dentin with 0.1 M to 0.5 M EDC or 5% GA for 60 seconds does not cause transdentinal cytotoxic effects on cultured odontoblast-like cells.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Effects of Multipurpose, Universal Adhesives on Resin Bonding to Zirconia Ceramic

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Clinical Relevance

Single Bond Universal and All-Bond Universal significantly improved the bond strength of resin cement to zirconia ceramic compared with Alloy Primer.

SUMMARY

This study evaluated the effects of single-bottle, multipurpose, universal adhesives on the bond strength of resin cement to zirconia ceramic. Polished zirconia ceramic (Cercon base) discs were randomly divided into four groups (n=40) according to the applied surface-conditioning agent: Single Bond 2, Single Bond Universal, All-Bond Universal, and Alloy Primer. Cured composite cylinders (Ø 0.8 mm × 1 mm) were cemented to the conditioned zirconia specimens with resin cement (RelyX ARC). The bonded specimens were subjected to a microshear bond-strength test after 24 hours of water storage and after 10,000 cycles of thermocycling. The surface-conditioning agent significantly influenced the bond strength ($p<0.05$). Single Bond Universal showed the highest initial bond strength (37.7 ± 5.1 MPa), followed by All-Bond Universal (31.3 ± 5.6 MPa), Alloy Primer (26.9 ± 5.1 MPa), and Single Bond 2 (8.5 ± 4.6 MPa). Artificial aging significantly reduced the bond strengths of all the test groups ($p<0.05$). After 10,000 cycles of thermocycling, All-Bond Universal showed the highest bond-strength value (26.9 ± 6.4 MPa). Regardless of artificial aging, Single Bond Universal and All-Bond Universal showed significantly higher bond strengths than Alloy Primer, a conventional metal primer.

INTRODUCTION

As patient demand for esthetic restorations has increased, zirconia ceramics have been frequently used as frameworks for metal-free restorations.[1] The

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development of computer-aided design and manufacturing technology has contributed to the popularity of zirconia ceramics as substitutes for dental metal alloys, which are generally processed by the lost wax technique. Zirconia restorations can be cemented with conventional cements because of their superior mechanical properties. However, a wide variety of clinical applications, such as partial coverage coronal restorations, Maryland-type resin-bonded fixed partial dentures, and intracoronal restorations, require a long-term durable bond to zirconia ceramics.

Numerous studies have proposed various methods for modifying the zirconia surface to improve resin bonding, including plasma spraying,[2] glass micro-pearls,[3] selective infiltration etching,[4] and a vapor phase deposition technique.[5] However, these methods require further investigation for clinical application. Although there is no consensus on the most suitable surface treatment method for zirconia, the combination of airborne-particle abrasion with Al_2O_3 particles for micromechanical interlocking and conditioning with a primer containing phosphate monomer for chemical bonding has been recommended.[6–10]

Commercially available surface-conditioning agents for zirconia generally contain functional monomers that are derived from the reaction of methacrylic acid with phosphoric or carboxylic acid.[9,11-13] One agent, 10-methacryloyloxydecyl dihydrogen phosphate (MDP; Figure 1), has been shown to provide chemical bonds between methacrylate-based materials and zirconia ceramics.[7,9,11,14] MDP was first introduced by Kuraray Medical Inc (Okayama, Japan) and has been included in the resin cements of Panavia, Alloy Primer, Clearfil Ceramic Primer, and Clearfil SE Bond. Recently, other manufacturers have introduced new MDP-containing adhesives to the dental market. These single-bottle adhesives are called "universal" adhesives because they can be used in etch-and-rinse or self-etch modes on the tooth substrates.[15,16] In addition, the manufacturers have suggested that these adhesives promote the bonding of methacrylate-based materials to various indirect restorative substrates, such as zirconia and dental non–precious metal alloys, with no need for an additional primer. However, little information is available about how these universal adhesives affect resin bonding to zirconia.

The purpose of this study was to evaluate the effects of single-bottle, multipurpose, universal adhesives on the bond strength of resin cement to zirconia ceramic compared with a conventional MDP-containing primer. The null hypothesis tested was that there would be no differences in bond strength or durability from a zirconia ceramic with different surface-conditioning agents. The bonded specimens were subjected to the microshear bond-strength test after 24 hours of water storage and after 10,000 cycles of thermocycling.

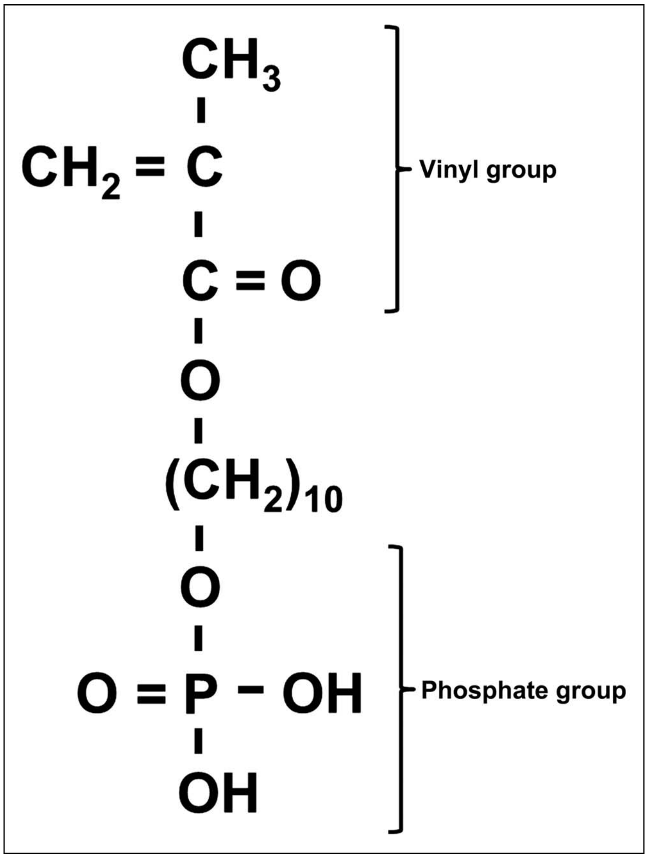

Figure 1. *Amphiphilic structure of the MDP monomer.*

METHODS AND MATERIALS

A partially sintered zirconia block (Cercon base, DeguDent, Hanau, Germany) was sectioned to produce 160 square specimens, which were then sintered according to the manufacturer's instructions. The fully sintered specimens (10 mm × 10 mm × 3 mm) were embedded in acrylic resin blocks. The zirconia surface was sequentially polished with up to 600-grit silicon carbide paper using an automatic polishing machine (Rotopol-V, Struers, Ballerup, Denmark) under water cooling and then underwent ultrasonic cleaning in isopropyl alcohol for 3 minutes. The specimens were randomly divided into four groups of 40 specimens each according to the applied surface-conditioning agent: Single Bond 2, Single

Table 1: *Surface Conditioning Agents and Resin Cement Used in This Study and Their Application Procedures*

Product (Batch No.)	Composition	Manufacturer	Application Procedure
Single Bond 2 (N412273)	bis-GMA, HEMA, DMA, methacrylate functional copolymer, filler, ethanol, water, photoinitiator	3M ESPE, St Paul, MN, USA	1. Apply the adhesive
			2. Allow it to react for 20 s
			3. Gently air dry for 5 s
Single Bond Universal (502225)	MDP, bis-GMA HEMA, DMA, methacrylate functional copolymer, filler, ethanol, water, initiators, silane	3M ESPE, St Paul, MN, USA	1. Apply the adhesive
			2. Allow it to react for 20 s
			3. Gently air dry for 5 s
All-Bond Universal (1200013674)	MDP, bis-GMA, HEMA, ethanol, water, initiators	Bisco Inc, Schaumburg, IL, USA	1. Apply the adhesive
			2. Air dry
			3. Light cure for 10 s
Alloy Primer (00436A)	VBATDT, MDP, acetone	Kuraray Medical Inc, Okayama, Japan	1. Apply the primer
			2. Leave it for drying
RelyX ARC resin cement (N441122)	bis-GMA, TEG-DMA, zirconia/silica filler, DMA, amine, photoinitiator, BP, pigment	3M ESPE, St Paul, MN, USA	1. Dispense the cement onto a mixing pad and mix for 10 s
			2. Apply a thin layer of the cement to the bonding surface

Abbreviations: bis-GMA, bisphenol A diglycidyl ethermethacrylate; BP, benzoyl peroxide; DMA, dimethacrylate; HEMA, hydroxyethyl methacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate; TEG-DMA, triethylene glycol dimethacrylate; VBATDT, 6-(4-vinylbenzyl-n-propyl amino)-1,3,5-triazine-2,4-dithione.

Bond Universal, All-Bond Universal, and Alloy Primer. The four different surface-conditioning agents used in this study are summarized in Table 1.

Polyethylene tubes (Tygon R-3603 tubing, Saint-Gobain Co, Courbevoie, France) were used to fabricate composite cylinders (0.8 mm in diameter and 1 mm in height). The tube was filled with composite resin (Filtek Z-250, 3M ESPE, St Paul, MN, USA) and light-polymerized from four directions for 20 seconds per side with a light-emitting diode (LED) curing unit (Elipar FreeLight 2, 3M ESPE). The light intensity of 800 mW/cm^2 was frequently monitored with a radiometer (Demetron 100, Demetron Research Co, Danbury, CT, USA). After light-polymerization, the composite cylinder was removed from the tube.

Three different MDP-containing agents were applied to the polished zirconia specimens strictly in accordance with the respective manufacturers' instructions as summarized in Table 1. Single Bond 2 was used as a negative control for the MDP-containing agents and applied according to the instructions of Single Bond Universal. Resin cement (RelyX ARC, 3M ESPE) was mixed and applied onto the composite cylinder, which was then placed on the zirconia specimen under a fixed load of 0.4 N. After excess resin cement was removed with a microbrush, glycerin gel was applied around the bonded interface. The resin cement was light-polymerized from four directions for 20 seconds per side with the LED curing unit. After 30 minutes at room temperature, the bonded specimens were stored in distilled water at 37°C for 24 hours. Next, 20 specimens of each group were immediately subjected to the bond-strength test. The remaining 20 specimens of each group were subjected to thermocycling for 10,000 cycles between 5°C and 55°C with a 25-second dwell time before the bond-strength test.

The microshear bond-strength test was performed with a universal testing machine (LF Plus, Lloyd Instruments, Fareham, UK). The experimental setup for the test is schematically shown in Figure 2. A stainless steel orthodontic wire (0.2 mm in diameter) was used to apply a shear force to the bonded interface. The wire, which was attached to the load cell, was looped around the composite cylinder as close as possible to the bonded interface. The shear force was applied at a crosshead speed of 0.5 mm/min until failure occurred.

The fractured interfaces of the specimens were examined with a stereomicroscope (SZ4045, Olympus Optical Co Ltd, Tokyo, Japan) at 40× magnification to determine the failure mode. The failure mode was classified as "adhesive failure" when it occurred between the zirconia ceramic and the resin cement and was classified as "mixed failure" when the adhesive failure and cohesive fracture occurred simultaneously within the resin cement. In cases of mixed failure, the surface of the zirconia was partly covered by the remaining resin cement.

Bond-strength data were analyzed using statistical software (SPSS 18.0, SPSS Inc, Chicago, IL, USA). One-way analysis of variance, followed by the

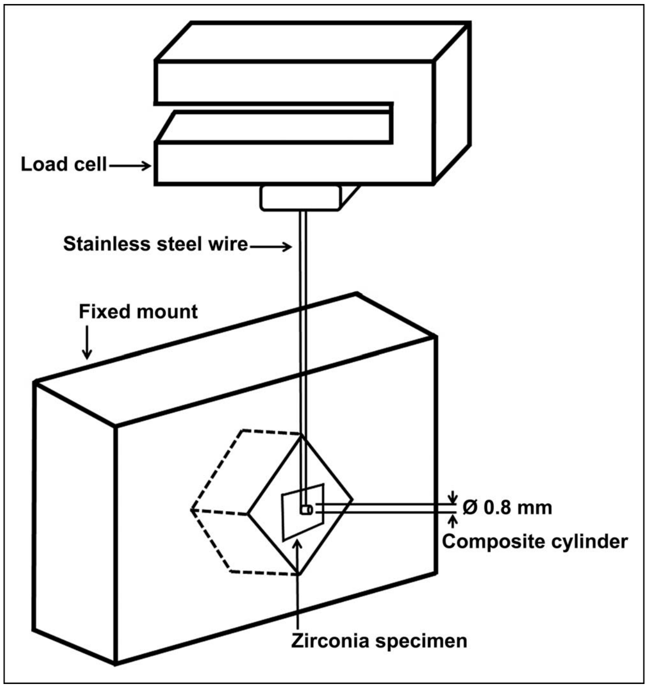

Figure 2. *Schematic drawing of the microshear bond-strength test.*

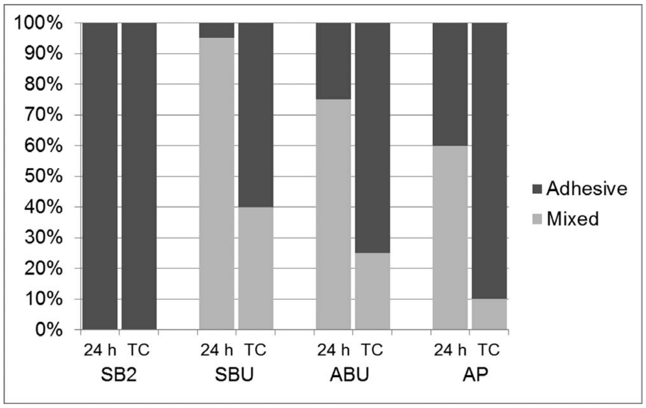

Figure 3. *Percentage distribution of failure modes after 24 hours or 10,000 cycles of thermocycling. Abbreviations: SB2, Single Bond 2; SBU, Single Bond Universal; ABU, All-Bond Universal; AP, Alloy Primer.*

Tukey honestly significant difference test for *post hoc* pairwise comparisons, was performed to assess the differences among the surface-conditioning agents. For each agent, the effect of thermocycling on bond strength was investigated using a two-sample *t*-test. The analyses were performed at a significance level of $\alpha=0.05$.

RESULTS

The mean bond-strength values and standard deviations are summarized in Table 2. Single Bond Universal, All-Bond Universal, and Alloy Primer significantly improved the bond strength of resin cement to zirconia compared with Single Bond 2 ($p<0.05$). The universal adhesives (Single Bond Universal and All-Bond Universal) showed significantly higher bond strengths than the conventional MDP-containing primer (Alloy Primer) regardless of the storage condition ($p<0.05$).

Before thermocycling, Single Bond Universal showed the highest bond-strength value ($p<0.05$). The bond strengths for all of the conditioning agents were significantly reduced after thermocycling ($p<0.05$). All-Bond Universal showed a significantly higher bond strength than Single Bond Universal after thermocycling ($p<0.05$).

The distribution of failure modes after the microshear bond-strength test is presented in Figure 3. Regardless of the storage condition, all of the specimens for Single Bond 2 were classified as adhesive failure after fracture. With the three MDP-containing agents, mixed failures (60% to 95%) predominated before thermocycling, but adhesive failures (60% to 90%) occurred more frequently than mixed failures (10% to 40%) after 10,000 cycles of thermocycling.

DISCUSSION

This study examined the effects of multipurpose, universal adhesives on the bond strength of resin cement to zirconia ceramic and compared them to

Table 2: *Means (Standard Deviations) of Microshear Bond Strength (in MPa) of Resin Cement to Zirconia Ceramic with Different Surface Conditioning Agents (n=20)*

Product	Feature of Agent	24 h[a]	10,000 Cycles of Thermocycling[a]
Single Bond 2	Conventional single-bottle adhesive	8.5 (4.6) $^{D}_{a}$	0.3 (0.1) $^{D}_{b}$
Single Bond Universal	MDP-containing single-bottle adhesive	37.7 (5.1) $^{A}_{a}$	20.7 (6.4) $^{B}_{b}$
All-Bond Universal	MDP-containing single-bottle adhesive	31.3 (5.6) $^{B}_{a}$	26.9 (6.4) $^{A}_{b}$
Alloy Primer	Conventional MDP-containing primer	26.9 (5.1) $^{C}_{a}$	10.7 (4.2) $^{C}_{b}$

Abbreviation: MDP, 10-methacryloyloxydecyl dihydrogen phosphate.
[a] *Different superscript uppercase letters indicate significant differences between data within the same column ($p<0.05$). Different subscript lowercase letters indicate significant differences between data within the same row ($p<0.05$).*

that of a conventional MDP-containing primer. Resin cements containing phosphate monomers have been shown to improve resin bonding to zirconia ceramic without any additional surface treatment.[6,8,17,18] Because the focus of the present study was on evaluating the performance of the MDP-containing adhesive agents, RelyX ARC, which does not contain any functional phosphate monomer, was selected as a luting cement and allowed for the differentiation of the effects of the surface-conditioning agents. The universal adhesives, namely Single Bond Universal and All-Bond Universal, produced higher initial bond strengths and maintained higher bond strengths after aging than did Alloy Primer.

The microshear bond-strength test was selected for this study. Various bond-strength tests, including shear,[2,3,8,9,13,14,19-21] tensile,[6,10] microshear,[18] and microtensile methods,[4,5,22] have been used to evaluate the effects of surface treatments on resin-zirconia bonding. Microtensile bond-strength tests allow for a more homogeneous distribution of stress and for the evaluation of the bond strength of a small region of interest within a substrate.[23] However, it is extremely difficult to section bonded zirconia specimens into microbeams without damaging the bonded interface because of the superior mechanical properties of zirconia. In addition, a high incidence of premature failure in the specimens has been reported with the microtensile bond-strength test, which would decrease the discriminative power of the test.[24]

Shear bond-strength tests have been widely used in the studies on resin-zirconia bonding.[2,3,8,9,13,14,19-21] However, the shear bond-strength test has been criticized for nonhomogeneous stress distribution at the bonded interface, inducing cohesive failures within the substrates and misinterpretations of the results.[25,26] However, these concerns were reduced because cohesive failures within zirconia have been rarely reported.[3,21] In the present study, the microshear bond-strength test allowed for the differentiation of the effects of the surface-conditioning agents with relatively small standard deviations. The microshear bond-strength test maximizes shear stresses at the bonded interface and gives precise results due to the reduced bonding area.[27] The test protocol for the microshear bond-strength test is simpler than that of the microtensile bond-strength test.

In the present study, the failure-mode distribution after the microshear bond-strength test was in line with the bond-strength data. All of the specimens for Single Bond 2, which showed the lowest mean bond strength, presented adhesive failures regardless of the storage condition. The specimens for the MDP-containing agents presented primarily mixed failures before thermocycling, whereas adhesive failures increased after thermocycling. The differences in the failure-mode distribution can be explained by the reduced bond strengths after thermocycling. Cohesive failures within the composite cylinders or the zirconia specimens were not observed, which implies that the interface between the resin cement and zirconia was the weakest link in the structure.

MDP chemically bonds to non–precious metals and tooth substrates.[28-30] MDP has an amphiphilic structure, with the vinyl group as the hydrophobic moiety and the phosphate group as the hydrophilic moiety (Figure 1). The vinyl group can copolymerize with the resin monomer of the resin-based materials applied later. MDP has also been shown to be effective in improving the resin bonding to zirconia ceramics.[6,8,10,11,13,14] It has been assumed that the hydroxyl groups of the phosphate moiety in MDP interact with the hydroxyl groups on the zirconia surface through Van der Waals forces or hydrogen bonds.[11] In the present study, the universal adhesives containing MDP showed significantly higher bond strengths than Single Bond 2. According to the manufacturer, Single Bond Universal differs from Single Bond 2 primarily in the addition of MDP and silane. There was a need to differentiate between the effects of conventional adhesive formulation and MDP on bond strength. For this, Single Bond 2 served as the negative control for the universal adhesives. Although Single Bond 2 showed the lowest bond strength among the groups, it seems that Single Bond 2 slightly improves the initial bond strength of RelyX ARC. In our preliminary test the bond strength of RelyX ARC to polished zirconia was almost zero without any primer or adhesive. The conventional adhesive formulation would allow the resin cement to flow more easily and adapt to the zirconia surface. However, the bond strength for Single Bond 2 was significantly lower than those of the universal adhesives. The higher bond strengths with universal adhesives can be explained by the addition of MDP to conventional adhesive formulations.

Single Bond Universal showed the highest initial bond strength. In contrast to All-Bond Universal, Single Bond Universal contains a silane in addition to MDP. The silane cannot contribute to the chemical bond to zirconia because zirconia lacks silica. However, the silane could increase the wettability of the zirconia surface and as a result improve the initial bond strength.[11,31] However, in spite of the high initial bond strength, Single Bond Universal showed a significantly lower bond strength than All-Bond Universal after thermocycling. The silane could increase the hydrophilicity of Single Bond Universal, thereby predisposing the adhesive layer to hydrolytic degradation.[32] This assumption needs further investigation.

Single Bond Universal and All-Bond Universal showed significantly higher bond strengths than Alloy Primer, a conventional MDP-containing primer. Although Alloy Primer was originally designed to enhance the bond between resin-based materials and dental metal alloys, it has provided a superior bond to zirconia compared with other primers containing phosphate monomer.[10,13,17] In contrast to Alloy Primer, the universal adhesives have resin adhesive components, which could allow the resin cement to flow more easily and strengthen the interfacial layer through copolymerizing with the resin cement. Based on the result of the present study, separate primers for conditioning the zirconia surface, such as Alloy Primer, can be substituted with these universal adhesives. The universal adhesives have also shown comparable performance on the tooth substrates compared with conventional adhesives.[15,16] The clinical procedure of cementing zirconia restorations could be simpler and more efficient with the single-bottle universal adhesives.

In the present study, the polished zirconia specimens were used without airborne-particle abrasion in order to focus on the role of the surface-conditioning agent. Airborne-particle abrasion is a crucial factor for a durable bond to zirconia.[6,7,10,13,14] Airborne-particle abrasion increases the surface roughness and area, thereby improving micromechanical retention. However, airborne-particle abrasion has a limited effect in improving the bond strength of resin cement to zirconia.[6,9,10] Another consideration is that airborne-particle abrasion with Al_2O_3 particles can induce phase transition and produce microcracks within the zirconia surface, which influence the mechanical properties of zirconia.[20,33,34] Özcan and others[34] reported that air abrasion with 50 μm Al_2O_3 particles at 2.8 bar pressure decreased the biaxial flexural strength of the zirconia. Large particles at a high blasting pressure increased surface roughness but did not result in a higher bond strength of resin cement to zirconia.[9,20,35] The initial bonding to zirconia seems to be mainly promoted by chemical bonds. However, previous long-term studies have shown that the chemical bonds are not water resistant,[6,7,10,13,14] which is in accordance with the present results. Therefore, airborne-particle abrasion, which has a surface activation and cleaning effect, is required to promote chemical bonds and increase bond durability.[10] Airborne-particle abrasion with silica-coated Al_2O_3 particles at reduced pressure has been recommended for producing a durable bond to zirconia with minimal influence on the mechanical properties of the material.[33,34,36] Further investigations that include various airborne-particle abrasion protocols should be conducted to achieve a durable bond to zirconia with the universal adhesives.

CONCLUSION

Within the limitations of the present study, the new universal adhesives showed better performance in terms of the bond strength of resin cement to zirconia ceramic compared with a conventional MDP-containing primer. Although artificial aging significantly reduced the bond strengths for all of the conditioning agents, the bond strengths of Single Bond Universal and All-Bond Universal remained higher than that of Alloy Primer. All-bond Universal presented the highest bond strength after thermocycling. The universal adhesives containing the MDP functional monomer could make the clinical procedure of cementing zirconia restorations simpler and more efficient.

Acknowledgement

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Conflict of Interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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The Effect of Sandblasting and Different Primers on Shear Bond Strength Between Yttria-tetragonal Zirconia Polycrystal Ceramic and a Self-adhesive Resin Cement

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Clinical Relevance

The combined use of air-abrasion and zirconia primer should be recommended for strong and durable bonding between yttria-tetragonal zirconia polycrystal and self-adhesive resin cement.

SUMMARY

Purpose: To evaluate the effect of zirconia primers, air-abrasion, and tribochemical surface treatment methods on the shear bond strength between yttria-tetragonal zirconia polycrystal (Y-TZP) ceramic and self-adhesive resin cement.

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Methods and Materials: Y-TZP ceramic surfaces were ground flat with 600-grit silicon carbide paper and then divided into seven groups of 10 and treated as follows: untreated (control), Monobond Plus, Z-PRIME Plus, ESPE Sil with CoJet, air-abrasion, Monobond Plus with air-abrasion, and Z-PRIME Plus with air-abrasion. Self-adhesive resin cement was placed onto the treated Y-TZP specimens for each group. All specimens were thermocycled and subjected to a shear bond strength test. Scanning electron microscope images of the fractured areas and x-ray diffraction (XRD) analysis of the surface-treated Y-TZP specimens were performed. Data were statistically analyzed using one-way analysis of variance and the Student-Newman-Keuls multiple comparison test ($p<0.05$).

Results: The Z-PRIME Plus treatment in combination with air-abrasion produced the highest bond strength (16.50±2.26 MPa), followed by air-abrasion (10.56±3.32 MPa), and then Monobond Plus combined with air-abrasion (8.93±3.13 MPa), ESPE Sil after CoJet application (8.54±3.98 MPa), and the Z-PRIME Plus group (8.27±2.79 MPa). The control (3.91±0.72 MPa) and Monobond Plus (4.86±1.77 MPa) groups indicated the lowest results ($p<0.05$). The XRD results showed the peaks of the monoclinic phase for the air-abrasion and CoJet treatment groups compared with the Y-TZP control.

Conclusion: Z-PRIME Plus primer application after air-abrasion presented the best results for improving the bond strength between Y-TZP ceramic and self-adhesive resin cement.

INTRODUCTION

All-ceramic restorations are a potentially more effective method compared with metal ceramic restorations in clinical situations requiring highly demanding esthetic restorations.[1] Due to its high fracture toughness and chemical durability, yttria-tetragonal zirconia polycrystal (Y-TZP) is one of the most frequently used materials today.[2,3]

However, the bond with Y-TZP is difficult to establish due to its acid-resistant and silica-free surface.[4] Hydrofluoric acid etching and silanization techniques have been unsuccessful for Y-TZP bonding and are only effective for glass ceramic materials.[5,6] Successful cementation of Y-TZP ceramic is crucial for the clinical success of the restoration, as is the case with any restorative material.[7,8]

For this reason, some manufacturers and researchers have attempted to modify the surface properties of Y-TZP using different methods. Tribochemical silica coating has been developed for zirconia bonding to increase the extent of micromechanical interlocking and chemical adhesion.[2,9,10] There is no conclusive evidence as to whether the silica becomes permanently attached to the Y-TZP.[4] It can, however, be predicted that forceful water rinsing will be able to remove temporarily attached silica; whereas, chemically bonded silica would be retained.[4] The introduction of surface roughening by airborne-particle abrasion has been suggested as an alternative method for high-strength ceramics.[11] Nevertheless, it is important to note that air-abrasion treatment introduces microcracks, which may be a significant degrading factor in terms of the long-term performance of Y-TZP restorations.[12,13]

If mechanical damage is to be avoided, alternative techniques to create a bond between the zirconia and the luting system would need to rely on a chemical interaction.[14] Various zirconia primers have been developed in an attempt to attain chemically improved adhesion between the resin cement and the dental Y-TZP.[3] These are easy to apply and do not require expensive and complicated apparatuses. However, controversial reports have emerged concerning their hydrolytic stability and have shown that zirconia primers are not always a favorable option.[2]

Because the technique of using multi-step resin cement is time consuming and complicated, self-adhesive resin cements have recently been developed. Despite increases in the clinical use of self-adhesive resin cements, further evidence regarding the adhesive cementation of Y-TZP restorations is necessary to establish the most reliable technique.[15] However, there is a lack of data on the shear strength of bonding to zirconia ceramics with the newly developed zirconia primers and self-adhesive resin cements.

Therefore, the aim of this study is to evaluate the effect of zirconia primers, air-abrasion, and tribochemical surface treatment methods on the shear bond strength between Y-TZP ceramic and self-adhesive resin cement. The null hypotheses tested suggested that different surface treatment applications do not influence the strength of the bonding to Y-TZP ceramic.

METHODS AND MATERIALS

Preparation of the Specimens

Partially sintered zirconia blocks of 97% zirconium dioxide (ZrO_2) stabilized with 3% yttria (Lava Frame,

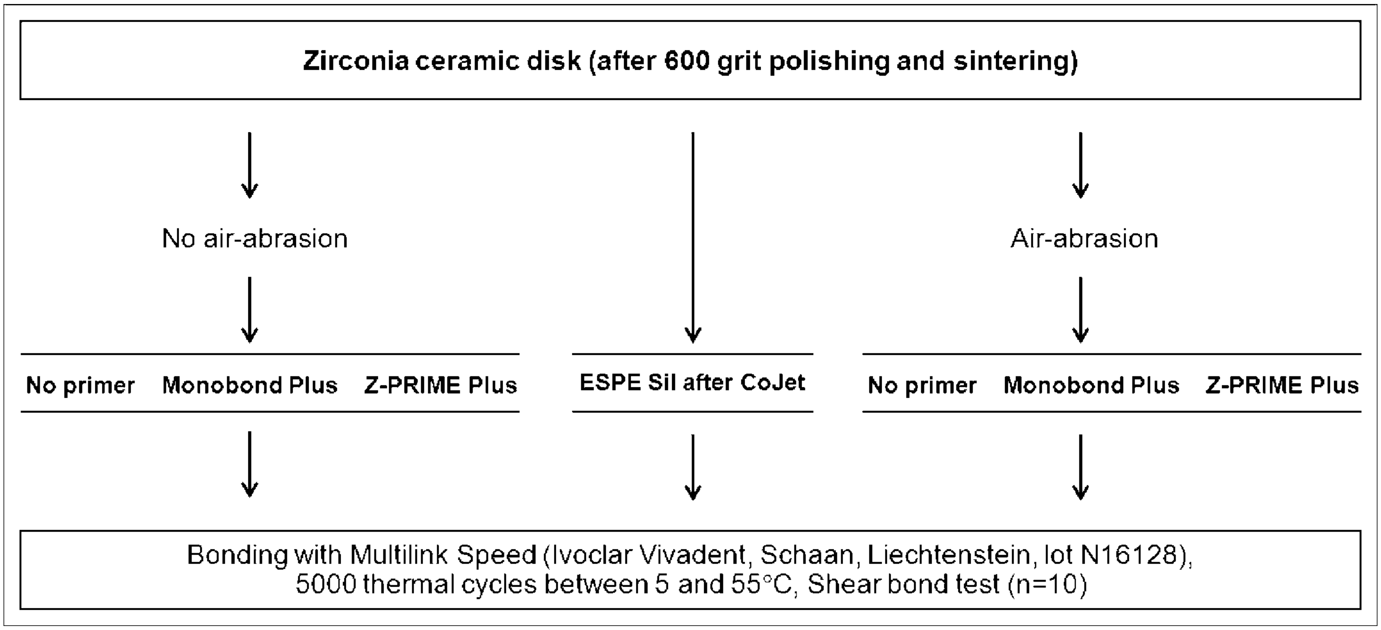

Figure 1. *Experimental design of the study.*

3M ESPE, St Paul, MN, USA), which were 19-mm in diameter and 100-mm high, were sectioned with a low-concentration diamond blade (Allied High Tech Productions Inc, Compton, CA, USA) to obtain 4-mm thick slices. The surfaces of each specimen were polished and ground with 600-grit silicon carbide abrasive using an underwater cooling method. The disk-shaped zirconia ceramics were ultrasonically cleaned in distilled water for three minutes, and then they were fully sintered using the manufacturer's instructions. Thereafter, they were embedded in polyethylene molds (19-mm inner diameter, 21-mm outer diameter, 12-mm height), with one side of the disk exposed for cement bonding.

Surface Treatments and Bonding Procedure

Subsequently, 70 specimens were randomly divided into seven groups according to the surface treatments and resin cement to be applied (N=70; n=10 per group). Figure 1 and Table 1 show the experimental design and the materials used in this study, respectively. Three of the groups were not subjected to air-abrasion and were directly treated with Monobond Plus (Ivoclar Vivadent, Schaan, Liechtenstein), Z-PRIME Plus (Bisco, Schaumburg, IL, USA), or did not undergo any primer treatment. Another three groups were subjected to air-abrasion with 50-µm grain size aluminum oxide (Al_2O_3) particles. Air-abrasion was conducted at a standoff distance of 10 mm with 3.5 bars of pressure for 15 seconds. The substrate surface was then rinsed for 30 seconds and air-dried for 30 seconds. The surface was then either treated with Monobond Plus or Z-PRIME Plus or not treated with any primer. The final group was subjected to tribochemical silica coating with the CoJet system (3M ESPE) using a 30-µm particle size. The substrate surface was then rinsed for 30 seconds and air-dried for 30 seconds.

Table 1: *Materials Used in the Study*

Brand Name	Composition	Batch Number	Manufacturer
CoJet	Silicatized sand (particle size, 30 µm) 30-42 psi 2-3 bar 15 seconds	368315	3M ESPE, St Paul, MN, USA
ESPE Sil	3-methacryloxyprophyltrimethoxy silane in ethanol	410841	3M ESPE, St Paul, MN, USA
Monobond Plus	Alcohol solution of silane methacrylate, phosphoric acid methacrylate and sulphide methacrylate	M35022	Ivoclar Vivadent, Schaan, Liechtenstein
Z-PRIME Plus	MDP, carboxlyate monomers	0900012783	BISCO, Schaumburg, IL, USA
Multilink Speed	Dimethacrylate, ytterbium trifluoride, adhesive monomer, MDP, glass, silicone dioxide	N16128	Ivoclar Vivadent, Schaan, Liechtenstein

Table 2: *Means and Standard Deviations for Shear Bond Strength Results (MPa) According to the Different Surface Treatments and Multilink Speed Cement*[a]

Priming Conditions	Surface Conditions[a]	
	No air-abrasion (polished)	Air-abrasion
None	3.91 (0.72)A	10.56 (3.32)B
Monobond-Plus	4.86 (1.77)A	8.93 (3.13)B
Z-PRIME plus	8.27 (2.79)B	16.50 (2.26)C
CoJet and ESPE Sil	8.54 (3.98)B	

[a] *Small caps that differ in each cell indicate statistical differences (p<0.05); whereas, identical small caps indicate no statistical difference in the designated group. Based on Student-Newman-Keuls multiple comparison test.*

The surface was then treated with a silane primer (ESPE Sil, 3M ESPE). Self-adhesive resin cement (Multilink Speed, Ivoclar Vivadent) was mixed according to the manufacturer's instructions and was placed inside a no. 5 size gel-cap (area=16.8 mm^2). The gel-cap was positioned on the zirconia surface and light-cured on four sides for 20 seconds per side using an LED light-curing unit (Eliper S10, 3M ESPE) at 600 mW/cm^2. For one hour at 23°C ± 1°C, the specimens were left to polymerize and then stored in distilled water for another 23 hours at 37°C. They were then subjected to 5000 thermal cycles between 5°C and 55°C. The dwelling time was 30 seconds at each temperature, and the transfer time between baths was two seconds. The pH values of the primers were measured by a pH electrode (Orion Glass Body ROSS Combination pH Electrode, Thermo Scientific, Beverly, MA, USA).

Bond Strength Test and Surface Analysis

The specimen was mounted in the jig of a universal testing machine (LF-Plus, Ametek Inc, Largo, FL, USA) and a load was applied to the adhesive interface (crosshead speed: 0.5 mm/min) until failure occurred. The corresponding software recorded the maximum stress (MPa) required to produce a failure. The failure mode was observed with a stereomicroscope (90×). An investigation of the resin bonding on the zirconia and fractured surfaces was carried out using a scanning electron microscope (SEM; S-4700 FESEM, Hitachi, Tokyo, Japan) at 150× and 3000× magnification and 10 kV accelerating voltage.

The formation of the monoclinic phase after air-abrasion and tribochemical surface treatments was determined with x-ray diffraction analysis (XRD; Diffractometer D5000, Siemens, Karlsruhe, Germany). The surfaces of the specimens were scanned with a copper Kα x-ray from 20° to 40° with a step size of 0.04° at five-second step intervals.

Statistical Analysis

The R programming language (R Foundation for Statistical Computing, Vienna, Austria)[16] was used to perform the data analysis. After checking the data normality and the equality of variance, a one-way analysis of variance (ANOVA) and a Student-Newman-Keuls multiple comparison test were performed. The mean difference was regarded as significant at the level of $p<0.05$.

RESULTS

The means and standard deviations for shear bond strength across all groups are presented in Table 2. A one-way ANOVA was used to calculate the statistical significance for the surface treatments ($p<0.05$). Air-abrasion, the use of a primer, and CoJet treatment were all more effective than the control group treatment, excluding the Monobond Plus treatment. The Z-PRIME Plus treatment after air-abrasion showed the best result.

The failure mode distribution for all samples is shown in Figure 2. The results showed a high percentage of adhesive failures at the zirconia surface in the control group. In contrast, when using additional surface treatments, the mixed failure mode was increased. All specimens presented mixed failure modes for the Z-PRIME Plus treatment group after air-abrasion.

Figures 3a-g show the representative SEM images (magnification, 150×) for Multilink Speed cement stubs on the contact area of the zirconia specimens. The remains of the resin cement can be seen to a relative degree (Figure 3b-g). It is notable that adhesive failure can be seen in the control group images (Figure 3a), yet the specimens treated with air-abrasion and Z-PRIME Plus exhibited mixed failure with resin cement and a characteristic corrugation appearance (Figure 3g).

Figure 4 shows the SEM images and energy dispersive spectroscopy (EDS) spectra for the surfaces conditioned with the CoJet system. A substantial number of silica particles could be seen on the zirconia surface after CoJet surface treatment. However, only a small amount of silica could be seen after the water rinsing, which indicates an air-abraded, roughened surface structure. The XRD results show the peak of the monoclinic phase in the case of sandblasting and the CoJet treatment groups compared with the Y-TZP control group

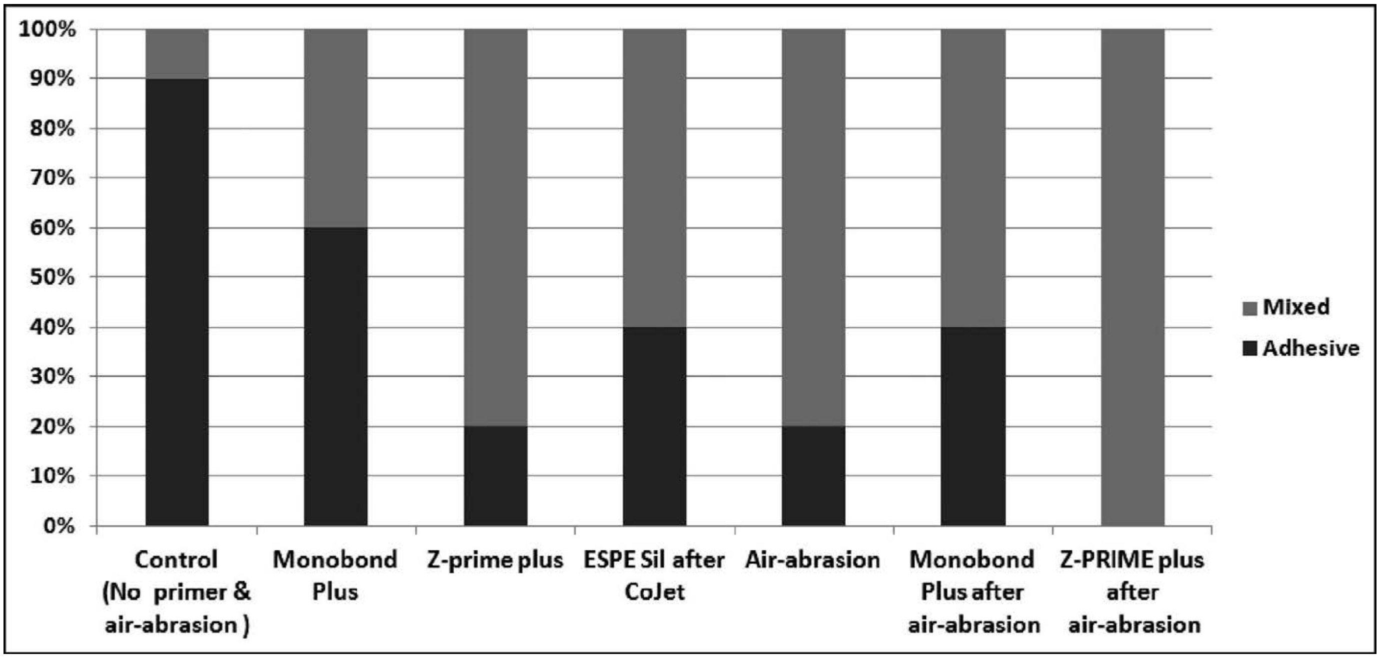

Figure 2. *Failure mode distribution.*

(Figure 5). The pH values of the primers were as follows, expressed in mean (SD): Monobond Plus, 1.63 (0.13); Z-PRIME Plus, 3.85 (0.04), and ESPE Sil, 4.39 (0.09).

DISCUSSION

This study investigated the effect of seven surface treatments on the shear bond strength of self-adhesive resin cement used on a Y-TZP ceramic. The results show that bonding was significantly affected by the air-abrasion, zirconia primer, and tribochemical silica coating treatments, and a combination of the air-abrasion method and the use of a zirconia primer showed the best result. Given that untreated zirconia surfaces presented the lowest bonding strength, the hypothesis of the study was nullified. This result is supported by previous studies that have shown that some conventional

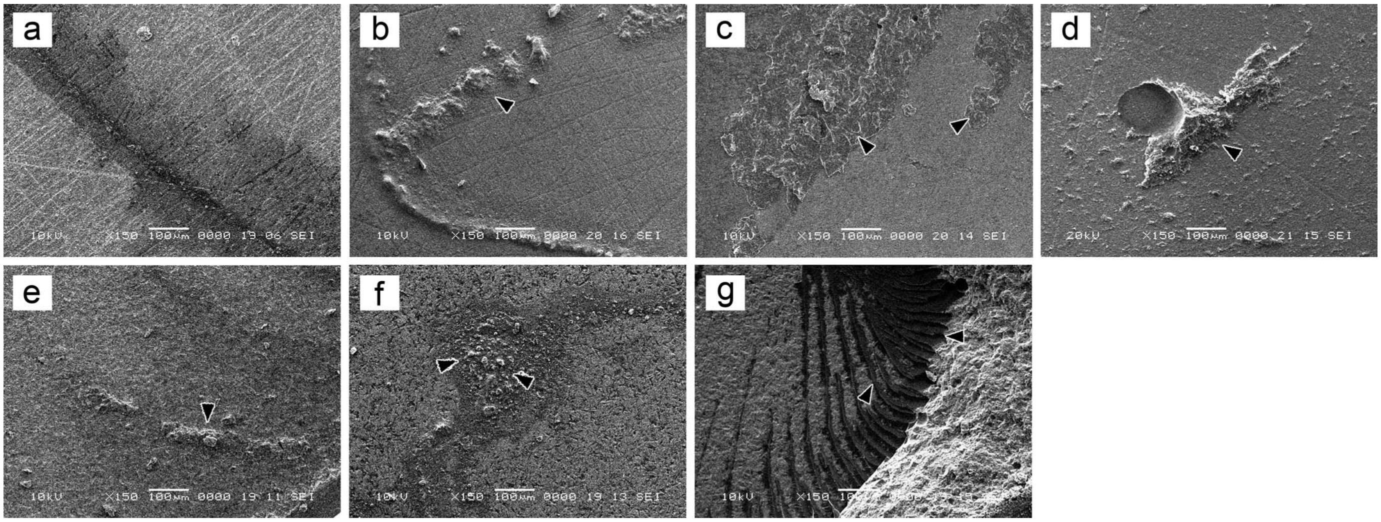

Figure 3. *Scanning electron micrographs (150× original magnification) of zirconia ceramic specimens cemented with Multilink Speed: (a) control surface without treatment; (b) surface coated with Monobond Plus; (c) surface coated with Z-PRIME Plus; (d) surface coated with ESPE Sil after tribochemical CoJet system treatment; (e) surface air-abrasion with 50 μm Al_2O_3; (f) surface coated with Monobond Plus after air-abrasion; and (g) surface coated with Z-PRIME Plus after air-abrasion. The arrows indicate the boundaries of the remaining resin cement.*

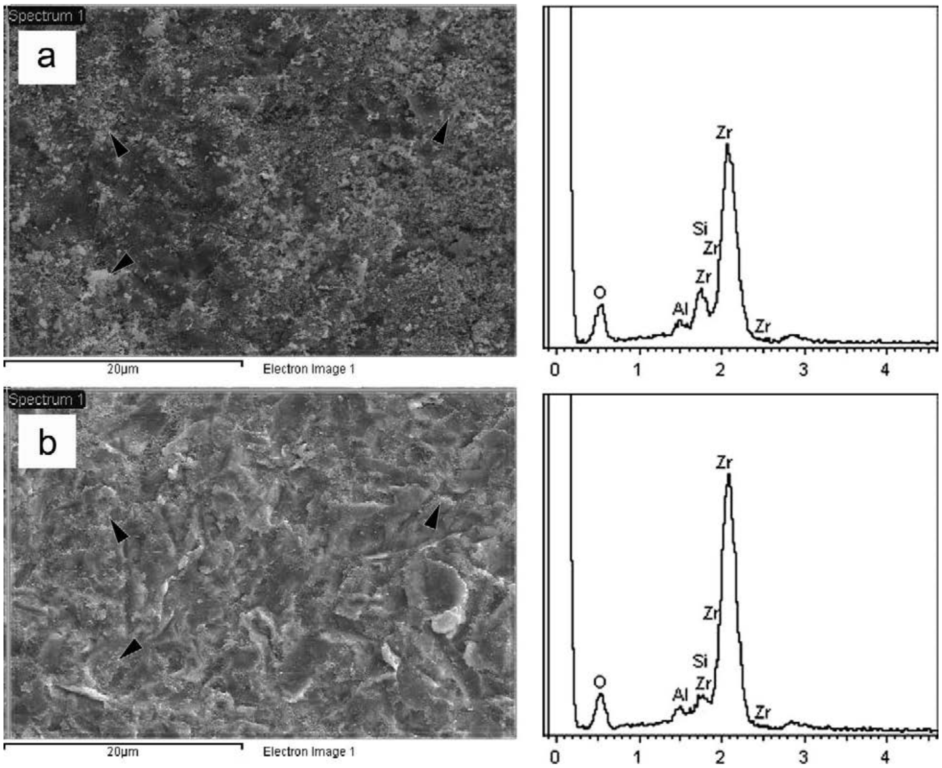

Figure 4. *SEM images (3000×) and EDS spectra: (a) surface conditioned with the tribochemical CoJet system; and (b) surface rinsed after tribochemical CoJet treatment. The small particles marked by the arrows indicate the remaining silica materials on the Y-TZP surface. The spectrum of the surface rinsed after the CoJet system treatment presents peaks of Al and Si. Zr, zirconium; Al, aluminum; O, oxygen; Si, silicon.*

and self-adhesive resin cements presented a low bonding strength when applied to an untreated zirconia ceramic surface.[17,18] These findings can be explained by the poor chemical interaction at the interfacial grain level between the hydroxyl groups of Y-TZP ceramics and the methacrylated phosphoric acid ester of self-adhesive resin cements such as Multilink Speed. Although this self-adhesive resin cement includes 10-methacryloyloxydecyldihydrogen phosphate (MDP), these phosphoric acid groups interacted chemically with ZrO_2,[17,19] and the functional monomer properties for the amount and flow appeared to be insufficient for increasing zirconia adhesion; the self-adhesive resin cement was insufficiently able to interact without any pretreatment.

The results showed that the bonding strength was significantly affected by 50-µm air-abrasion, regardless of whether the specimens were pretreated with zirconia primers. This result is in good agreement with those of previous studies.[17,18,20] The increase in roughness and surface energy that resulted from the air-abrasion method may have eased the resin cement flow into the microretention, thereby increasing the micromechanical interlocking between the resin cements and zirconia surfaces.[2,18,20] Moreover, an increased surface area on the Y-TZP would have been generated by air abrasion, increasing the chemical reaction with the phosphate groups of Multilink Speed as well as improving the micromechanical interlocking.

Two primers of different compositions were used in this study. Treating the Y-TZP surface with Z-PRIME Plus significantly increased the shear bonding strength for the intact zirconia surfaces and showed the best bonding strength result when combined with the air-abrasion pretreatment. Z-PRIME Plus contains two adhesive functional monomers (carboxylic and MDP monomers) that interact chemically with the zirconium oxide layer at the resin and zirconia interfaces. It is possible that these interfacial forces could have improved the surface wettability and chemical affinity of Y-TZP ceramics, thus increasing the interlocking with the self-adhesive resin cement. The synergic effect of the acidic MDP and carboxylate monomers is the most likely explanation for the higher bonding strength seen with this primer.[21] The bonding strength for Z-PRIME Plus surface treatment after air-abrasion was twice that when only the Z-PRIME Plus primer was applied. In this group, the failure mode results

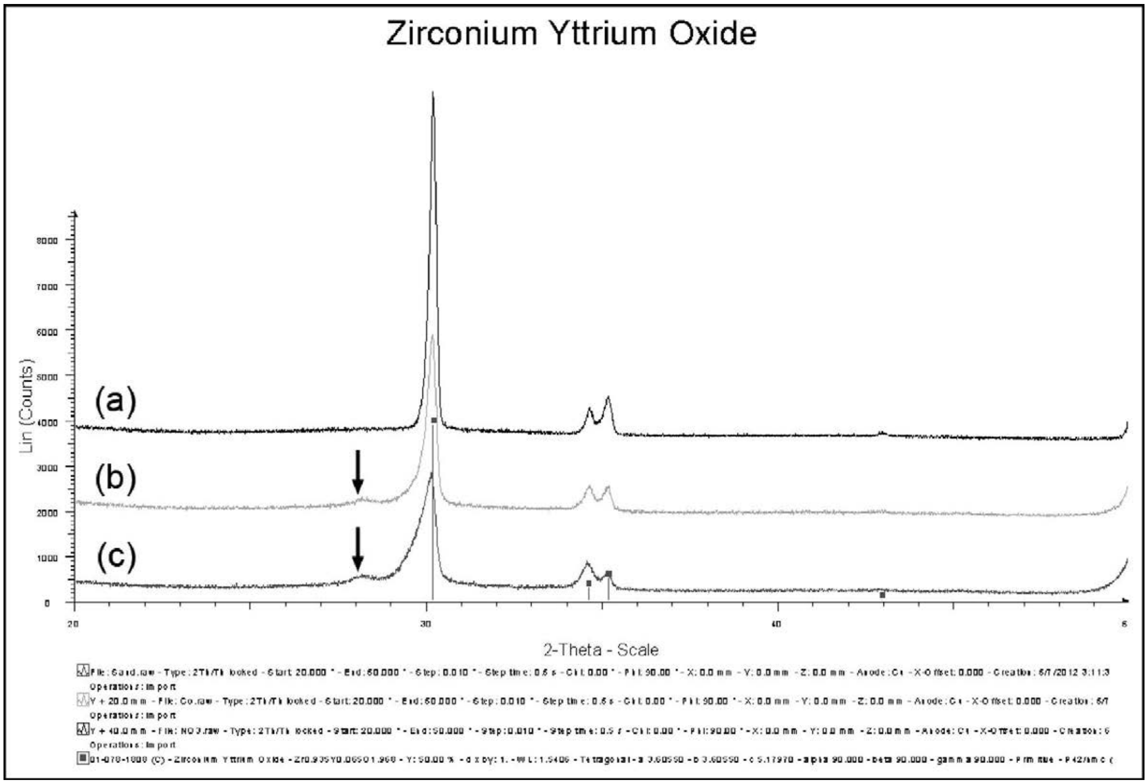

Figure 5. *XRD results after air-abrasion and CoJet treatment on zirconia surfaces: (a) control; (b) surface coated with 30 μm silica-modified Al_2O_3 particles; and (c) surface air-abrasion with 50 μm Al_2O_3. The arrows indicate monoclinic phase transformation.*

showed all mixed fracture patterns. This was due to a synergic effect produced by the increased contact area on the chemical interactions between the MDP and carboxylic monomers of the Z-PRIME Plus and the Y-TZP ceramic surface. Representative SEM images of this group and the air-abrasion group showed a unique corrugated fracture appearance, a mixed fracture pattern, and a thicker primer layer compared with the images for the samples treated with Monobond Plus primer. This corrugated appearance is considered to indicate the phenomenon of strong adhesion to resist the shear force. However, the Y-TZP surface treatment with the Monobond Plus primer did not significantly increase the shear bonding strength for the intact zirconia surfaces and showed adhesive and mixed failure modes. For the group with Monobond Plus application after air-abrasion pretreatment, the results showed that the bonding strength significantly increased compared with the control group, although this seems to have been a result of the air-abrasion rather than the primer. SEM images for the Monobond Plus group showed a thinner layer compared with those for the Z-PRIME Plus group and did not show a unique fracture appearance regardless of whether there was air-abrasion. Monobond Plus consists of silane methacrylate for glass ceramic, sulfide methacrylate for precious metal alloys, and phosphoric acid methacrylate for oxide ceramics and base metal alloys. Zirconia ceramic is composed of a glass-free material, which means that the silane cannot take effect. Monomers containing sulfur from 6-methacryloxyhexyl 2-thiouracil-5-carboxylate (MTU-6) and 6-[N- (4-vinylbenzyl) propylamino]-1,3,5-triazine 2,4-dithione (VBATDT) are purposely chosen to promote the adhesion to noble alloys; however, for bonding to base metal alloys, they are not always effective. Likewise, Monobond Plus has a low pH value of 1.63 because the phosphate monomer is acidic.[22,23] As such, some water and the silane component in acidic alcohol may be hydrolyzed. The silane priming ability may be reduced by polymerizing via condensation reaction to form the polysiloxane oligomer, and therefore there may be interference with zirconia bonding ability.[24,25]

Using the tribochemical CoJet system, the ceramic surface is abraded with silica-modified Al_2O_3 particles, leading to silica inclusions in the ceramic surfaces. A chemical bond between the organic resin materials and the silica-modified zirconia surfaces may be formed after silica-coating due to the silanization that occurs with a silane coupling agent.[26] However, there is a lack of consensus about whether the silica is chemically bonded to zirconia or merely weakly attached. The tribochemical modification mechanism where silica attaches to a densely sintered and mechanically tough zirconia surface is still not well understood.[21] However, achieving a stable and permanent attachment is generally perceived as extremely important in the wider aim of achieving strong and durable bonds to zirconia. In the present study, SEM, EDS, and a shear bond strength test were used to investigate whether the tribochemical coating/silane treatment was effective for improving zirconia bonding. Both the SEM and EDS data confirmed that the silica nanoparticles were easily removed simply by rinsing with a forceful water stream. ESPE Sil application after the water rinsing did not improve the bonding strength. Indeed, this bonding strength was only similar to the result for the air-abrasion group, which most likely explains why the 30-μm silica particle size increased the surface roughness of the zirconia surface.

Phase transformation of Y-TZP may be caused by air-abrasion, which will create microcracks. Unfavorable changes to the superior mechanical properties of the material may be caused.[27,28] Although the bonding strength was significantly affected by air-abrasion and tribochemical silica coating due to this method increasing the surface contact area, the representative phenomenon of phase transformation was observed in the XRD results. Zirconia is prone to low temperature degradation due to the metastability of its phase transformation from a tetragonal to a monoclinic phase. The long-term effects of phase transformation on zirconia ceramics still requires further research.

CONCLUSIONS

Within the limitations of the current experimental settings, it can be concluded that the application of self-adhesive resin cement without pretreatment was not sufficient for improving the strength of the bonding to an untreated zirconia ceramic surface. The combined use of the air-abrasion method and a zirconia primer appears to be a reliable method of achieving strong and durable bonding between zirconia ceramics and self-adhesive resin cements. It should also be noted that using air-abrasion with a 50-μm particle size and a CoJet application of 30-μm particle size can lead to a phase transformation on the Y-TZP surface.

Acknowledgements

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Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Penetration of Hydrogen Peroxide and Degradation Rate of Different Bleaching Products

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LTÂ Cintra • RC Pascotto • PH dos Santos
ALF Briso

Clinical Relevance

During in office bleaching techniques, the reapplication of products is not necessary.

SUMMARY

This study's aim was to evaluate the degradation rate of hydrogen peroxide (H_2O_2) and to quantify its penetration in tooth structure, considering the residence time of bleaching products on the dental enamel. For this study, bovine teeth were randomly divided according to the bleaching product received: Opalescence Xtra Boost 38%, White Gold Office 35%, Whiteness HP Blue 35%, Whiteness HP Maxx 35%, and Lase Peroxide Sensy 35%. To analyze the degradation of H_2O_2, the titration of bleaching agents with potassium permanganate was used, while the penetration of H_2O_2 was measured via spectrophotometric analysis of the acetate buffer solution, collected from the artificial pulp chamber. The analyses were performed immediately as well as 15 minutes, 30 minutes, and 45 minutes after product application. The data of degradation rate of H_2O_2 were submitted to analysis of variance (ANOVA) and Tukey tests, while ANOVA and Fisher tests were used for the quantification of H_2O_2, at the 5% level. The results showed that all products significantly reduced the concentration of H_2O_2 activates at the end of 45 minutes. It was also verified that the penetra-

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tion of H_2O_2 was enhanced by increasing the residence time of the product on the tooth surface. It was concluded that the bleaching gels retained substantial concentrations of H_2O_2 after 45 minutes of application, and penetration of H_2O_2 in the dental structure is time-dependent.

INTRODUCTION

The smile transmits most of a person's characteristics and expressions, thus influencing self-esteem and social relationships. In recent years, the search for cosmetic procedures has increased significantly, especially those that involve tooth whitening. Thus, tooth whitening has become a main demand for patients in dental clinics and has been incorporated as an essential procedure in most treatment plans that involve the esthetics of a smile.[1,2]

However, although bleaching vital teeth is considered to be a conservative treatment and is biologically safe, concerns still arise regarding the use of highly concentrated products, as their uses are related to structural changes in dental tissues and postoperative sensitivity.[3-7] Thus, a good professional should know the indications of the different bleaching agents, the advantages of each technique, and the potential effects on teeth.

Currently, the most common product used in the in-office bleaching technique is based on 35% and 40% hydrogen peroxide and is applied under the supervision of dental professionals. Although variations exist in the method of application for different products, this treatment requires multiple clinical sessions in order for an esthetic result to be obtained.[8,9] Although few studies assess the real need for reapplication during the same session, the professional usually replaces the whitening gel multiple times, continually renewing the product that is in contact with the tooth structure.

Despite the fact that the success of bleaching is directly related to the diffusion capacity of the peroxides, it is believed that successful bleaching is not related to continuous reapplication because color change is clinically observed even when reapplication is not performed.[10]

Studies that evaluate the concentration of peroxide in bleaching gels over time show continuous liberation of peroxide even after 15 minutes of application,[11-14] which indicates the real possibility of a bleaching product to maintain its bleaching capacity, even without reapplication.

Additionally, exposure to excessive and unnecessary bleaching products increases the levels of hydrogen peroxide in the pulp tissue, which could cause lipid peroxidation, cell membrane injury, and even cell death.[15,16] Given these facts, it is necessary to measure the concentration and quantify the penetration of hydrogen peroxide through the dental tissue during its contact with the enamel.

It is believed that this knowledge can contribute to the refinement of bleaching therapy, as clinicians are employing significant resources in order to offer the best whitening treatments for their patients, though they may be providing unnecessary exposure to peroxides.[3-6,17]

Therefore, this present study evaluated the degradation rate of hydrogen peroxide and quantified its penetration into the tooth structure when considering different application times.

METHODS AND MATERIALS

Factors Under Study

1. Bleaching products based on hydrogen peroxide at five levels: Opalescence Xtra Boost 38% (Ultradent, South Jordan, Utah, USA), White Gold Office 35% (Dentsply Ind Com Ltda, Petrópolis, RJ, Brazil), Whiteness HP Blue 35% (FGM Dental Products, Joinville, SC, Brazil), Whiteness HP Maxx 35% (FGM Dental Products), and Lase Peroxide Sensy 35% (DMC Equipment, São Carlos, SP, Brazil).
2. Time after placement at four levels: immediately, 15 minutes, 30 minutes, and 45 minutes after placement.

The response variables included the concentration of hydrogen peroxide in bleaching products as well as the transenamel and transdentinal penetration of hydrogen peroxide. The three basic principles of experimentation (repetition, randomization, and blocking) were respected.

Solution Preparation and Analysis of the Degradation Rate of Hydrogen Peroxide

Five syringes of each bleaching product were used to verify the decomposition of hydrogen peroxide at different times (Table 1). The method used is based on titration with potassium permanganate.[18,19] This method describes the amount of hydrogen peroxide in a bleaching product. This method is based on a reduction-oxidation reaction, according to the following formula: $2KMnO_4 + 5H_2O_2 + 4H_2SO_4 = 2KHSO_4 + 2MnSO_4 + 5O_2 + 8H_2O$.

Table 1: *Characteristics of the Materials Studied*

Group	Trademark	%[a]	Manufacturer/Lot	Manipulation
G1	Opalescence Xtra Boost	38%	Ultradent/B6BSV	Two coupled syringes, one containing the peroxide and the other with the activator
G2	White Gold Office	35%	Dentsply/675123E	Two coupled syringes, one containing the peroxide and the other with the activator
G3	Whiteness HP Blue	35%	FGM/240712	Two coupled syringes, one containing the peroxide and the other with the activator
G4	Whiteness HP Maxx	35%	FGM/290812	Phase I mixed with phase II at a ratio of a measure of powder to a liquid droplet
G5	Lase Peroxide Sensy	35%	DMC/30403	Phase I mixed with phase II at a ratio of a measure of powder to a liquid droplet

[a] Hydrogen peroxide concentration given by the manufacturer.

The solution of potassium permanganate (solution 1) was prepared by mixing 0.2 g of sodium oxalate, 250 mL of distilled water, and 15 mL of sulfuric acid at 80°C for 30 minutes. Then, the solution was kept in an amber glass that was protected from light for 24 hours.

The bleaching agent was mixed in accordance with each manufacturer's instructions and evaluated immediately and at 15 minutes, 30 minutes, and 45 minutes after mixing. During this period, the bleaching products were kept on a glass plate. In order to obtain the volume of hydrogen peroxide in a sample, the bleaching agents were analytically weighted, and a sample of approximately 2 mg of each bleaching product was collected and diluted in 10 mL of distilled water (solution 2). Then, solution 1 was added to solution 2 at a rate of 0.1 mL/sec until a violet color was observed. This color change indicated the equivalence point, ie, the moment when all of the H_2O_2 had been consumed. The volume of solution 1 required to change the color of the solution was applied to the following formula: $C = V \times Cf \times 1.701 \times 100m$, where

- C = hydrogen peroxide concentration (w/w);
- V = volume of solution 1 in milliliters added during titration;
- Cf = correction factor for the solution of 0.1 N potassium permanganate;
- m = mass of the bleaching product sample in milligrams.

Preparation and Standardization of Specimens

Seventy-five experimental specimens were obtained from bovine incisors taken from animals aged between 24 and 30 months. Analysis of enamel specimens was performed using stereomicroscopy (Stemi SV11, Carl Zeiss Microscopy, Thornwood, NY, USA) at 45×. Those specimens presenting with morphologic changes and/or the presence of cracks in the enamel were excluded.

The selected teeth were mechanically cleaned with periodontal curettes and received prophylaxis with pumice and water. Subsequently, the specimens were fixed on a device attached to a drill platform (FGC-16 model, Ferrari, São Paulo, SP, Brazil). Cylinders were obtained from the middle third of the buccal surface (5.7 mm in diameter) using a cylindrical diamond-cutting instrument designed to cut glass (diamond tip, 8 mm in diameter, Dinser Diamond Tools Ltda, Sacomã, SP, Brazil) under constant irrigation.

The dentin surface was smoothed using manual rotation with 600-grit aluminum oxide sandpaper (T469-SF-Noton, Saint-Gobam Abrasives Ltda, Jundiai, SP, Brazil) until the specimens presented a thickness of 3.5 mm (approximately 1.3 mm and 2.2 mm of enamel and dentin, respectively), as measured with a digital caliper (500 to 144 B, Mitutoyo South America Ltda, Suzano, SP, Brazil). The smear layer formed during grinding was removed by applying EDTA for 1 minute. The specimens were rinsed with deionized water.

Division of the Groups

After the manufacture of the specimens, they were divided into five groups (n=15) according to Table 1.

Preparation of Artificial Pulp Chamber

Each enamel/dentin disc was individually adapted to an artificial pulp chamber (APC), developed at the Laboratory of Experimental Pathology and Biomaterials of Araraquara School of Dentistry – Uni Estadual Paulista (UNESP).[17] Each APC was formed by two compartments: the upper portion with an 8-mm diameter opening and the lower

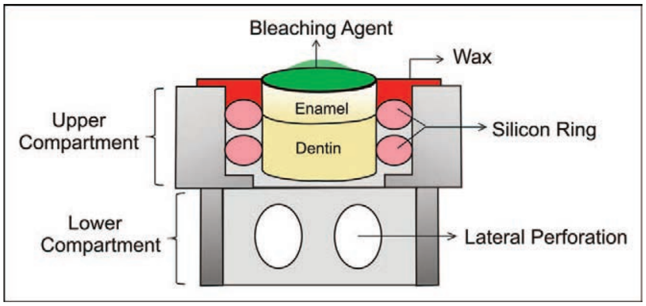

Figure 1. *Representative design of an enamel/dentin disc placed in an APC.*

portion with a 6-mm diameter opening, allowing the appropriate positioning and lateral sealing of the specimen. The lower portion presenting lateral perforations to circulate the acetate buffer solution was used to quantify the peroxide penetrating the specimen.

The specimens were positioned in the APCs between two silicone rings (5.60-mm inner diameter, 1.78-mm thick, Ref. OR 008, Rodimar Rolamentos Ltda, Araraquara, SP, Brazil) and sealed with pink wax, restricting the lateral penetration of the bleaching agent (Figure 1).

Whitening Procedure

Three in-office bleaching sessions were performed at weekly intervals, using 0.04 mL of each bleaching product (Table 1). All procedures were performed in accordance with each manufacturer's instructions.

Preparation of Solutions and Transenamel and Transdentinal Penetration of H_2O_2

In order to quantify the H_2O_2 that penetrated the enamel/dentin discs, the artificial pulp chambers were individually placed in the wells of acrylic plates for cell culturing. Each well was filled with 1 mL of acetate buffer solution and subsequently received APCs that already contained dental fragments. Thus, the dentin surface remained in contact with the acetate solution during all bleaching procedures and the diffused hydrogen peroxide became part of the acetate buffer solution.

After the whitening procedures, 25 μL of acetate buffer solution was removed and mixed with 2750 μL distilled water, 100 mL leucocrystal violet (0.5 mg/mL, Sigma Chemical Co, St Louis, MO, USA) and 50 μL peroxidase (1 mg/mL, Sigma Chemical Co). The resultant solution was diluted to a final volume of 3 mL using distilled water.

Quantification of H_2O_2 Penetration

The quantification of hydrogen peroxide was carried out 15, 30, and 45 minutes after the placement of the bleaching product.

This method is based on the reaction of hydrogen peroxide with leucocrystal violet, as catalyzed by the peroxidase enzyme.[20] The coloring of the mixture varies in intensity according to the amount of peroxide. Thus, as the color value is proportional to the absorbance of the peroxide concentration, it is possible to indirectly assess the amount of peroxide that penetrated the tooth surface and the solution contained in the wells.

Readings were performed using Ultraviolet Visible reflectance spectrophotometric equipment (UV-2450, Shimadzu, Kyoto, Japan), 30 minutes after each bleaching session.

To obtain the calibration factor (CF) equivalent to the ratio of the concentration of the standard solution of hydrogen peroxide to its absorbance, the following equation was used: $CF = [Sample\ Solution]/Absorbance$.

The average calibration factor was used to calculate the hydrogen peroxide concentration contained in each specimen. Data were tabulated and the normality and homogeneity of variance assumptions were verified. Parametric tests were performed using analysis of variance (ANOVA) and Fisher test with the statistical program Stat View software at a significance level of 0.05.

RESULTS

The application of ANOVA and post-hoc Tukey test showed that all products significantly reduced the active concentration of H_2O_2 over time. Furthermore, the comparison among the products showed that Whiteness HP Maxx gel had the lowest concentration of hydrogen peroxide when compared to the other products, independent of the time of analysis. On the other hand, Opalescence Xtra Boost had the highest active concentration of the product. The mean values of the degradation rates over time are summarized in Table 2.

The application of ANOVA and Fisher test for the analysis of the data related to the transenamel and transdentinal penetration of hydrogen peroxide indicated that, in general, the penetration of H_2O_2 was intensified by increasing the dwell time of the product on the tooth surface ($p<0.0001$) (Figure 2).

Little variation was found in the penetration of hydrogen peroxide among the different products.

Table 2: *Mean Peroxide Concentrations (%) of the Degradation of the Bleaching Gel Over Time**

Products	0 Minutes	15 Minutes	30 Minutes	45 Minutes
Opalescence Xtra Boost	35.94 (0.62)Aa	35.49 (0.88)Aa	34.73 (0.17)Aab	33.80 (0.82)Ab
White Gold Office	35.21 (0.42)Aa	33.56 (1.20)ABb	32.15 (0.87)Bbc	30.79 (0.35)Bc
Whiteness HP Blue	34.67 (0.76)Aa	33.27 (0.93)Bb	32.10 (0.33)Bbc	31.11 (0.45)Bc
Whiteness HP Maxx	32.45 (0.71)Ba	30.97 (1.73)Cab	29.99 (0.89)Cbc	28.84 (0.60)Cc
Lase Peroxide Sensy	34.48 (1.37)Aa	33.36 (0.46)Bab	32.11 (0.75)Bb	29.94 (0.87)BCc

** Statistical significance is denoted by letters: upper-case letters in the columns and lower-case letters in the rows indicate significant differences between the products and time, respectively ($p<0.05$).*

However, Lase Peroxide Sensy 35%, Opalescence Xtra Boost 38%, and White Gold Office 35% penetrated more deeply when compared to the other products during the first 30 minutes. When evaluating the time of 45 minutes, Whiteness HP Blue 35% and Lase Peroxide Sensy 35% presented the greatest penetration, while Whiteness HP Maxx presented the lowest results (Figure 2; Table 3).

DISCUSSION

The use of bovine teeth allowed us to obtain experimental specimens with suitable dimensions to establish artificial pulp chambers in order to create an experimental model similar to the clinical application of bleaching agents on dental enamel during a session of in-office whitening. It is important to highlight that bovine teeth also present a uniform composition and have low variations in experimental responses.[21-25] However, human teeth are morphologically and histologically similar to bovine incisors.[26-28]

The use of peroxide in high concentrations (35%-38%) allows for noticeable bleaching results after the first bleaching session. This has been the main marketing appeal of in-office bleaching, which has increased the popularity of this technique in recent years.[29] However, reports of many and varied side effects of this treatment are common.[23,30,31] Thus, several authors have directed their research to the study of effective doses that would not cause postoperative sensitivity or other harmful effects on dental tissues.[32-35]

In this current study, the hydrogen peroxide was quantified from different products using a standard formula. This methodology has been used for a long time[11,13,19,29] and is widely used in the pharmaceutical industry (United States Pharmacopeia)[18] and allows for the estimation of the concentration of active products present in different bleaching gels.

White Gold Office and Whiteness HP Blue were the only products that presented an initial active concentration of H_2O_2 (±0.5%) that was indicated by the manufacturer. Moreover, all of the products revealed a significant reduction of hydrogen peroxide at the end of the 45-minute application. Lase Peroxide Sensy had the highest reduction in active

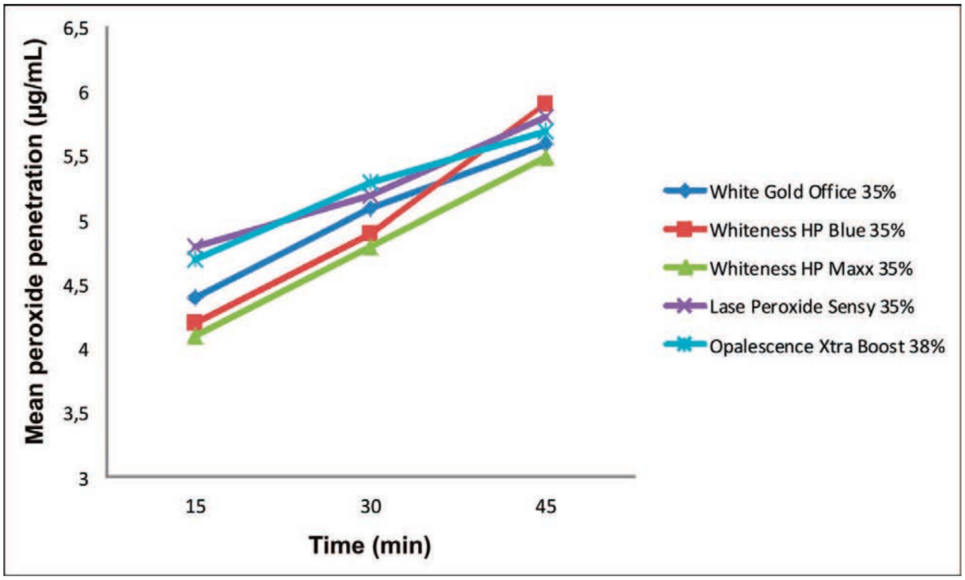

Figure 2. *Mean (µg/mL) of trans-enamel and transdentinal penetration of hydrogen peroxide obtained from artificial pulp chamber after bleaching, according to the different application times and products.*

Table 3: *Mean Peroxide Concentrations (μg/mL) Recovered From Artificial Pulp Chambers After Tooth Whitening, According to the Different Application Time and Products**

Time, min	White Gold Office 35%	Whiteness HP Blue 35%	Whiteness HP Maxx 35%	Lase Peroxide Sensy 35%	Opalescence Xtra Boost 38%
15	4.4 (0.5)Cabc	4.2 (0.6)Cbc	4.1 (0.7)Cc	4.8 (0.6)Ca	4.7 (0.4)Ca
30	5.1 (0.5)Bac	4.9 (0.3)Bbc	4.8 (0.3)Bb	5.2 (0.5)Ba	5.3 (0.3)Ba
45	5.6 (0.2)Abc	5.9 (0.3)Aa	5.5 (0.5)Ab	5.8 (0.3)Aac	5.7 (0.2)Abc

** Statistical significance is denoted by letters: upper-case letters in the columns and lower-case letters in the rows indicate significant differences between time and the products, respectively ($p<0.05$).*

concentration of H_2O_2 when considering the initial concentration. However, this reduction may be considered small as more than 86% of the initial concentration of the product remained. These data suggest that the bleaching gels in the current study retain their whitening ability and maybe did not need to be reapplied during the sessions, thus contradicting many bleaching procedures. The present results are in opposition to those obtained by Al-Qunaian and others,[11] Matis and others,[13] and Wattanapayungkul and others,[36] where *in vivo* analysis verified that some bleaching gels used in at-home bleaching techniques exhibited intense degradation after a few hours of their applications. However, it should be emphasized that higher temperatures are found in the *in vivo* condition, and the constant contact of the saliva with the bleaching products could intensify the degradation of peroxides. This increased temperature did not occur in the experimental conditions of this study.

The results of the transenamel and transdentinal penetration of the H_2O_2 released by bleaching agents showed that all products presented rapid diffusion through the enamel and dentin, as also reported in other studies.[21,37-39] It was also verified that penetration of hydrogen peroxide is time-dependent. In other words, longer contact times of the bleaching gel with the enamel structure provides greater penetration of the peroxide through dental tissues. These results corroborate the findings of Soares and others,[40] who evaluated the effectiveness of bleaching gels on enamel after different application periods. Similar responses were also obtained by Camargo and others[38] and Bowles and Ugwuneri,[3] with both studies concluding that the diffusion of hydrogen peroxide through enamel and dentin is related to the contact time of the bleaching agent with the enamel.

When making a general analysis of the results obtained in the current study and considering that the bleaching gels substantially retained their initial concentrations of hydrogen peroxide, it was expected that the penetration quantified after a 45-minute application would be three times higher than that obtained after a 15-minute application. However, the current results showed that the penetration does not increase as a function of time. Similar results were obtained by Soares and others[40] when measuring H_2O_2 following different application periods on the dental enamel.

Thus, a single application of the bleaching product can produce results that are similar to those protocols already preestablished and evaluated with regard to the availability of H_2O_2 in a bleaching product.[41] In this context, a single application would involve a reduction in the time required to complete the clinical procedure, reduce the cost, and lessen the risk of accidents that involve the soft tissues.

However, other factors that are related to the safety of the bleaching procedures should be considered and studied. The pH variation of the product during its application can cause concern if it reaches values that are considered to be critical, where it may cause significant histomorphologic changes of the enamel surface.[42,43] On the other side, it has been reported that products with neutral pH do not alter enamel surface roughness, even after multiple applications.[44]

These results cannot be extrapolated directly to a clinical situation, since the current study is an *in vitro* study and did not include the presence of intrapulpal pressure, the presence of saliva, or cytoplasmic processes of odontoblasts that may decrease the peroxide diffusion.[4,45-48] Even so, the results of the current study strongly indicate the adoption and improvement of in-office bleaching protocols to include single applications of the bleaching product.

CONCLUSIONS

Under the conditions of the current study, it can be concluded that:

- Bleaching gels maintain more than 86% of their initial concentration of hydrogen peroxide after 45 minutes.

- The penetration of hydrogen peroxide in the tooth structure is time-dependent.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Effect of Different Matrix Metalloproteinase Inhibitors on Microtensile Bond Strength of an Etch-and-Rinse and a Self-etching Adhesive to Dentin

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Clinical Relevance

Matrix metalloproteinase (MMP) inhibitors prevented bond strength loss of an etch-and-rinse adhesive over time. Bond strength of a self-etching adhesive was not significantly reduced with 9 months of aging; thus, MMP inhibitors did not show a significant effect.

SUMMARY

Aim: This study aimed to analyze the effect of different matrix metalloproteinase (MMP) inhibitors on the microtensile bond strength (microTBS) of an etch-and-rinse and a self-etching adhesive after 9 months of aging.

Methods and Materials: Flat human dentin surfaces were bonded either with an etch-and-rinse adhesive (Optibond FL) or a self-etching adhesive (Clearfil SE Bond). Dentin surfaces were left untreated or were pretreated with MMP inhibitors (2% chlorhexidine digluconate [CHX], 0.05% green tea extract, 1 mM ferrous sulfate, or 0.2 mM galardin) prior to application of the adhesive. Composite buildups were made incrementally. Pretreated groups were tested after 9 months of storage in artificial saliva (37°C) and compared with untreated groups, which were tested immediately (initial microTBS) and upon aging (9-month microTBS). Data were analyzed by linear mixed-model regression. Failure mode analysis was performed microscopically and statistically analyzed by repeated-measures analysis of variance (p<0.05).

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Results: MicroTBS of the etch-and-rinse adhesive but not of the self-etching adhesive was significantly decreased by aging. For Optibond FL, pretreatment with 2% CHX, 0.05% green tea extract, and 0.2 mM galardin revealed bond strength values (MPa) similar to the initial microTBS (32.1±14.8) and significantly higher compared with the microTBS (20.3±13.6) of aged untreated dentin. No significant differences were observed between groups bonded with Clearfil SE Bond (initial microTBS: 28.3±12.4; 9-month microTBS: 25.3±11.8). Application of the MMP inhibitors decreased the number of adhesive failures compared with untreated controls after 9 months of aging, but this effect was not significant.

Conclusion: The MMP inhibitors prevented the decrease in microTBS upon aging of the etch-and-rinse but not of the self-etching adhesive.

INTRODUCTION

Matrix metalloproteinases (MMPs) and cysteine cathepsins in dentin and dentinal fluid contribute to the enzymatic degradation of the adhesive hybrid layer and thus to the reduction of bond durability over time. pH changes caused by acid etching or acidic monomers and the adhesive resin monomers themselves can modulate the activation and expression of MMPs and cysteine cathepsins, resulting in an increased digestion of collagen within the hybrid layer.[1,2]

Different strategies have aimed to improve the bond durability by applying enzyme inhibitors as a pretreatment before resin infiltration or by admixing enzyme inhibitors to primers. It has been shown that chlorhexidine digluconate (CHX) can inhibit[3] MMP-2, −8, and −9 and of cysteine cathepsins,[4] preserving bond strength of etch-and-rinse and self-etching adhesives over time.[5-7]

As the application of 2% CHX on phosphoric acid-etched dentin is the only application procedure that has been tested clinically and shown to prevent bond strength loss *in vivo*,[8-10] this procedure can be considered as a kind of gold standard for maintaining hybrid layer stability. However, in recent studies, other potential MMP inhibitors, such as galardin, metal ions, or green tea catechins, were tested, which were suggested to be as or even more effective than CHX.[11,12] Similar to CHX, galardin as a synthetic inhibitor of MMP-1, −2, −3, −8, and −9 is acting as a zinc chelator.[13] Green tea catechins, mainly epigallocatechin-gallate (EGCG), change the secondary structure of collagenases by hydrogen bonding and hydrophobic interactions.[14] As well, binding of metal ions might cause conformational changes that inactivate their catalytic function.[15]

However, to date, the efficacy of these MMP inhibitors to prevent dentin bond strength loss over time has not been determined. Thus, the aim of the present study was to investigate the microtensile bond strength (microTBS) of an etch-and-rinse (Optibond FL) and a self-etching adhesive (Clearfil SE Bond), both of which have been shown repeatedly to be excellent adhesives in their respective class, after pretreatment with different MMP inhibitors.

This study tested the null hypothesis that the microTBS of an etch-and-rinse and a self-etching adhesive after 9 months of storage in artificial saliva is not affected by pretreatment with water-based solutions containing 2% CHX, 0.05% green tea extract, 1 mM $FeSO_4$, or 0.2 mM galardin.

METHODS AND MATERIALS

Specimen Preparation

Forty extracted, sound human molars were selected for this study. The teeth were stored in 0.01% (w/v) thymol at 4°C and were used within four weeks after extraction. Extracted teeth were collected as anonymous by-products of regular therapy. Because of that, our Medical Ethical Board stated that the performed research did not fall under the regulations of the "Act on Medical Research Involving Human Subjects" (METc 2009.305).

The teeth were embedded in circular molds with self-curing acrylic resin (Paladur, Heraeus Kulzer, Germany). A flat, mid-coronal dentin surface was prepared by means of a water-cooled, low-speed diamond saw (Isomet 1000, Buehler Ltd, Lake Bluff, IL). Polishing with wet 600-grit SiC paper (Water Proof Silicon Carbide Paper, Stuers, Erkrat, Germany) created a standardized smear layer. The dentin surfaces were verified for the absence of pulp chamber exposition using a stereomicroscope (Stemi 2000, Carl Zeiss, Feldbach, Switzerland).

The teeth were allocated randomly to test (each n=3) and control (n=5) groups of the two adhesives.

Bonding Procedure

Bonding of dentin surfaces was performed using an etch-and-rinse (Optibond FL, Kerr, Scafati, Italy) or a self-etching adhesive (Clearfil SE Bond, Kuraray, Tokyo, Japan). The two adhesives were applied

Table 1: *Manufacturer, Composition, and Application Procedure of the Adhesive Systems*

Adhesive/Manufacturer	Batch Number/Composition	Application Procedure
Optibond FL, Kerr, Scafati, Italy	Primer (LOT: 3490336): HEMA, ethanol, GPDM, MMEP, water, CQ, BHT Adhesive (LOT: 3502324): Bis-GMA, HEMA, GDMA, CQ, ODMAB, approximately 48wt% filled	Dentin conditioning: 37% H_3PO_4 (15 s) Water rinsing (15 s) Gentle air drying (5 s) Test groups only: MMP inhibitor solution (60 s) Excess removal Adhesive application: Primer (15 s) Gentle air drying (5 s) Adhesive (15 s) Light curing (20 s)
Clearfil SE Bond, Kuraray, Okayama, Japan	Primer (LOT: 00997A): HEMA, 10-MDP, hydrophilic aliphatic dimethacrylate, CQ, water, accelerators, dyes Bond (LOT: 011482A): Bis-GMA, HEMA, 10-MDP, hydrophobic aliphatic methacrylate, colloidal silica, QC, initiators, accelerators	Test groups only: MMP inhibitor solution (60 s) Excess removal Adhesive application: Primer (20 s) Gentle stream (evaporation) Adhesive (20 s) Light curing (10 s)

Abbreviations: 10-MDP, 10-methacryloyloxydecyl dihydrogen phosphate; BHT, butylhydroxytoluene; Bis-GMA, bisphenol A diglycidyl methacrylate; CQ, camphorquinone; GDMA, glycerol dimethacrylate; GPDM, glycerol phosphate dimethacrylate; HEMA, 2-hydroxyl methacrylate; MMEP, mono-2-methacryloyloxyethyl phthalate; MMP, matrix metalloproteinase; ODMAB, 2-(ethylhexyl)-4-(dimethylamino)benzoate.

according to the manufacturer's instructions (Table 1) with or without the additional application of one of the different MMP inhibitor solutions: 1) CHX (2%), 2) green tea extract (0.05%), ferrous sulfate (1 mM), or 4) galardin (0.2 mM; Table 2).

To analyze the initial microTBS and the bond strength after aging (microTBS after 9 months), two groups of each adhesive were not treated with any MMP inhibitor. The remaining groups were treated with one of the MMP inhibitors and tested only after aging, as the immediate bond strength between the untreated controls and test groups was shown to be not different.[11,20]

The application of the MMP inhibitor solutions was performed with a microbrush for 60 seconds under a slight rubbing motion. The excess was removed using an absorbing paper, leaving the dentin surface moist.

After light-curing of the bonding, a composite buildup (CeramX Mono, A3, Dentsply, Konstanz, Germany) was made in five 1 mm increments, each light-cured for 40 seconds at 800 mW/cm^2 (bluephase, IvoclarVivadent, Schaan, Liechtenstein).

MicroTBS Test

After 24-hour storage in water at 37°C, the teeth were sectioned perpendicular to the interface with a water-cooled diamond saw (Struers-Accutom 50, Struers, Denmark, and MOD 10, Struers, Denmark) to obtain rectangular beams of approximately 1 mm^2. The interfaces were precisely checked under a stereomicroscope to examine whether enamel remained. Sticks with remaining enamel were discarded, while all other sticks from one tooth were used. The beams of the control group were tested immediately (initial microTBS), while the beams of test groups were stored in artificial saliva[21] at 37°C for 9 months. Thereby, all sticks from one tooth were stored in 3 mL of artificial saliva.

Table 2: *Manufacturers and Concentrations of the Water-Based Matrix Metalloproteinase (MMP) Inhibitor Solutions*[a]

MMP Inhibitor/Manufacturer	Concentration of MMP Inhibitor Solution	Reference
Chlorhexidine digluconate, Kantonsapotheke, Zurich, Switzerland	2 wt%	5,16
Green tea extract, OM24, Omnimedica, Zurich, Switzerland	0.05 wt%	14,17
$FeSO_4$, Merck, Darmstadt, Germany	1 mM	12,18
Galardin, Merck, Darmstadt, Germany	0.2 mM	11

[a] *MMP inhibitors at the respective concentrations were chosen according to studies demonstrating a reduction in dentin degradation[12,16] or MMP-2 and/or MMP-9 activity.[5,11,18,19]*

Table 3: *Microtensile Bond Strength (MPa, mean±standard deviation), Number of Specimens Tested (n), Pretesting Failures (pf), and Failure Distribution (%) in the Different Groups*[a]

Adhesive	Group	Aging	MicroTBS, MPa	n	pf	Failure Distribution, %			
						A	M	CD	CC
Optibond FL	Control	No (initial testing)	32.1 ± 14.8*	68	15	22.1	64.7	4.4	8.8
	No treatment	9 mo	20.3 ± 13.6	40	2	42.5	52.5	0	5.0
	CHX	9 mo	32.9 ± 11.3*	35	0	31.4	51.4	5.7	11.4
	GTE	9 mo	33.2 ± 14.0*	41	0	31.7	53.7	7.3	7.3
	$FeSO_4$	9 mo	25.3 ± 10.5	32	0	43.8	43.8	6.2	6.2
	Galardin	9 mo	33.6 ± 10.5*	35	0	42.9	45.7	2.8	8.6
Clearfil SE Bond	Control	No (initial testing)	28.3 ± 12.4	69	12	13.0	75.4	4.3	7.2
	No treatment	9 mo	25.3 ± 11.8	37	0	46.0	43.2	5.3	5.3
	CHX	9 mo	32.9 ± 11.3	41	1	36.6	53.7	2.4	7.3
	GTE	9 mo	26.1 ± 14.2	38	1	26.3	57.9	5.3	10.5
	$FeSO_4$	9 mo	25.3 ± 10.5	30	1	40.0	50.0	0	10.0
	Galardin	9 mo	33.6 ± 14.1	36	1	38.9	55.6	2.8	2.8

Abbreviations: A, adhesive; CC, cohesive in composite; CD, cohesive in dentin; CHX, chlorhexidine digluconate; GTE, green tea extract; M, mixed.

[a] *Groups marked by an asterisk were significantly different from the group without pretreatment.*

The artificial saliva contained 0.7 mmol/L $CaCl_2$, 0.2 mmol/L $MgCl_2 \cdot 6H_2O$, 4.0 mmol/L KH_2PO_4, 30 mmol/L KCl, 0.3 mmol/L NaN_3, and HEPES buffer (all reagents purchased by Merck, Darmstadt, Germany) and was renewed weekly.

The tensile load of the beams was tested in a universal testing machine (Z010, Zwick/Roell, Ulm, Germany) with a 200 N load cell (KAF-TC, A.S.T., Dresden, Germany) at a crosshead speed of 0.15 mm/min. Therefore, the nontrimmed beams were fixed to a sandblasted microtensile bond jig with cyanoacrylate glue (Model Repair II Blue, Dentsply-Sankin, Tochigi, Japan) and tested under tensile force in a top-bottom manner described previously.[22] The dimensions of the beams were measured with a digital caliper to calculate the exact cross-sectional area.

The bond strength values (MPa) were determined by dividing the imposed force (N) at the time of fracture by the bond area (mm^2).

In addition, each beam was observed under a stereomicroscope (Wild M8, WILD HEERBRUGG, Heerbrugg, Switzerland) at 50× magnification to determine the mode of failure, classified as adhesive (A), cohesive in dentin (CD), composite (CC), or mixed (M).

The number of sticks and pretest failures in each group is given in Table 3.

Statistical Analysis

The mean microTBS (±standard deviation) for each group was computed. Kolmogorov-Smirnov and Shapiro-Wilk tests were applied to check the assumption of normality. Normal distribution was found in all groups, and data were further analyzed by linear mixed-model regression, separately for each adhesive, to account for the fact that multiple beams were gained from the same tooth and, therefore, were not independent from each other.

Relative frequencies of adhesive, mixed, and cohesive failures were calculated and analyzed by repeated-measures analysis of variance (ANOVA) followed by Greenhouse-Geyser correction using the adhesive and the test groups as independent covariables. As no significant differences with respect to the adhesives were found, differences between the test groups were statistically analyzed by one-way ANOVAs and Scheffé post hoc tests separately for each failure type.

The level of significance was set at $p<0.05$.

RESULTS

The microTBS of the etch-and-rinse adhesive was significantly decreased after 9 months of storage in artificial saliva ($p=0.01$). Pretreatment with all MMP inhibitors except $FeSO_4$ revealed bond strength values similar to the initial microTBS and significantly higher compared with the aged group without pretreatment (9-month microTBS, $p<0.013$).

The microTBS of the self-etching adhesive declined only slightly but not significantly over time ($p=0.49$). Bond strength values after pretreatment with the different MMP inhibitors were not different from the initial microTBS nor from the 9-month microTBS data ($p>0.359$; Table 3).

The failure mode analyses revealed significant differences between the test groups for adhesive ($p=0.003$) and mixed ($p=0.04$) but not for cohesive failures (dentin: $p=0.749$; composite: $p=0.528$). Overall, the frequency of adhesive failures in groups without MMP inhibitors was significantly ($p=0.008$) increased by aging. Application of the MMP inhibitors decreased the number of adhesive failures, but this difference was not significant with respect to the untreated groups after 9 months of aging ($p>0.34$). However, the relative frequencies of adhesive failures after pretreatment with CHX and green tea extract were not significantly ($p>0.07$) different from the untreated groups tested immediately. Post hoc comparisons of mixed failures showed no significant differences between the test groups ($p>0.061$).

DISCUSSION

The null hypothesis that the microTBS after 9 months of storage in artificial saliva is not affected by pretreatment with different MMP inhibitors was corroborated for the self-etch adhesive but not for the etch-and-rinse adhesive. Pretreatment with all MMP inhibitors except $FeSO_4$ prevented microTBS loss of Optibond FL significantly and equally effectively.

In the present study, the MMP inhibitor solutions were applied in a separate step in addition to the three-step or two-step application procedure of the etch-and-rinse or the self-etching adhesive, respectively. This approach might increase the overall chair time for a composite restoration and might be less feasible for self-etching adhesives because of the synchronous demineralization and infiltration during treatment. However, the application time of the MMP inhibitor solutions was limited to 60 seconds, which seems realizable under clinical conditions. Moreover, the incorporation of MMP inhibitors into dental adhesives might affect the mechanical properties of the products by decreasing the degree of conversion and the E-modulus.[23]

The initial microTBS found for Optibond FL and Clearfil SE Bond correlates with the results found previously.[24,25] Both adhesives have consistently shown favorable bonding performance in different protocols for microTBS measurement. As the bonding performance of Optibond FL and Clearfil SE Bond was shown to be hardly affected by the cavity configuration[26] and dentin location,[25] the setup of the present study was simplified by using flat dentin surfaces. Flat dentin surfaces allowed for the use of multiple sticks per tooth; thus, the overall sample size could be increased. The sticks were aged by direct exposure to artificial saliva, which is a quicker aging strategy than aging intact bonded teeth.[27]

Both adhesives exhibited lower microTBS values after aging, but this effect was significant only for the etch-and-rinse adhesive. Moreover, the fracture analysis revealed predominantly mixed adhesive-cohesive failure patterns with a clear tendency to fail more at the interface after 9 months of storage in the case in which no MMP inhibitor was applied. These results are in accordance with previous studies indicating a more rapid destruction of hybrid layers for three-step etch-and-rinse adhesives compared with mild two-step self-etching adhesives.[20,25] Although adhesive hydrophilicity and water sorption of adhesive interfaces are still considered the principal mechanisms of the resin-bond degradation, enzymatic degradation of the hybrid layer by MMPs contributes to the degradation process and loss of bond strength over time. The higher levels of MMP-2 and MMP-9 activity demonstrated for etch-and-rinse compared with self-etching adhesives[2,20] might therefore partly explain why the bond durability of Optibond FL is affected to a higher extent than that of the Clearfil SE Bond. However, it is likely that with longer aging times, the effect of enzymatic degradation might become more apparent for Clearfil SE Bond as well.[20]

For both adhesives, application of the MMP inhibitors resulted in microTBS values similar to the initial bond strength. It is likely that this effect is related to the reduction of enzymatic dentin degradation. In a previous study, the degradation of demineralized dentin after application of 0.012% CHX, 400 μM EGCG, and 1 mM $FeSO_4$ was directly determined by assaying hydroxyproline. All test agents reduced enzymatic collagen degradation distinctly but were not significantly different from each other.[12]

As the catechin concentration in the green tea extract solution is about 30%,[28] the inhibitory doses reported for EGCG and ECG on MMP-2 and MMP-9 were distinctly exceeded.[14] EGCG concentrations between 0.02% and 0.5% were shown to prevent bond strength loss of an etch-and-rinse adhesive over 6 months,[29] thereby being as effective as a 2% chlorhexidine solution. Because of the low toxicity and the anti-inflammatory potential, polyphenol catechins can be considered as very biocompatible, with the MMP inhibitor as a possible rewetting agent.

Pretreatment with the galardin solution was as effective as chlorhexidine in preserving bond strength over time, which supports the results of a previous study, in which 0.2 mM galardin solution was used as rewetting agent for an etch-and-rinse adhesive.[11] In contrast to these results, galardin-modified adhesives at lower concentration were shown to be unable to prevent bond strength loss after three months of storage time.[30]

Although ferrous sulfate was applied in a concentration previously shown to inhibit MMP-2 and MMP-9 activity and to reduce erosive dentin loss probably by inhibiting enzymatic degradation of the collagenous dentin matrix,[18] it was unable to inhibit bond strength loss significantly. The inhibitory action of ferrous sulfate on MMPs might be partly abrogated by the fact that precipitates formed on the dentin surface and in the dentin tubules[18,31] act as a mechanical barrier hampering penetration of the adhesive. Moreover, ferric sulfate might induce some dentin discoloration,[18] which might affect the color of a composite restoration negatively.

CONCLUSIONS

The MMP inhibitors chlorhexidine, green tea extract, and galardin preserved the microTBS of Optibond FL but not of Clearfil SE Bond significantly when aged for 9 months in artificial saliva. In the case of ferric sulfate application, bond strength was not affected negatively over time. It remains to be determined whether these MMP inhibitors will prevent bond strength loss in long-term experiments.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Influence of Light Intensity on Surface Free Energy and Dentin Bond Strength of Core Build-up Resins

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Clinical Relevance

The surface free energy and dentin bond strength of the dual-cure core build-up systems tested in this study were affected by the light intensity of the curing unit. When using dual-cure core build-up systems, practitioners need to consider the light intensity of the curing unit to achieve the optimal bond strength.

SUMMARY

Objective: We examined the influence of light intensity on surface free energy characteristics and dentin bond strength of dual-cure direct core build-up resin systems.

Methods: Two commercially available dual-cure direct core build-up resin systems, Clearfil DC Core Automix with Clearfil Bond SE One and UniFil Core EM with Self-Etching Bond, were studied. Bovine mandibular incisors were mounted in acrylic resin and the facial dentin surfaces were wet ground on 600-grit silicon carbide paper. Adhesives were applied to dentin surfaces and cured with light intensities of 0 (no irradiation), 200, 400, and 600 mW/cm^2. The surface free energy of the adhesives (five samples per group) was determined by measuring the contact angles of three test liquids placed on the cured adhesives. To determine the strength of the dentin bond, the core build-up resin pastes were condensed into the mold on the adhesive-treated dentin surfaces according to the methods described for the surface free energy measurement. The resin pastes were cured with the same light intensities as those used for the adhesives. Ten specimens per group were stored in water maintained at 37°C for 24 hours, after which

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they were shear tested at a crosshead speed of 1.0 mm/minute in a universal testing machine. Two-way analysis of variance (ANOVA) and a Tukey-Kramer test were performed, with the significance level set at 0.05.

Results: The surface free energies of the adhesive-treated dentin surfaces decreased with an increase in the light intensity of the curing unit. Two-way ANOVA revealed that the type of core build-up system and the light intensity significantly influence the bond strength, although there was no significant interaction between the two factors. The highest bond strengths were achieved when the resin pastes were cured with the strongest light intensity for all the core build-up systems. When polymerized with a light intensity of 200 mW/cm^2 or less, significantly lower bond strengths were observed.

Conclusions: The data suggest that the dentin bond strength of core build-up systems are still affected by the light intensity of the curing unit, which is based on the surface free energy of the adhesives. On the basis of the results and limitations of the test conditions used in this study, it appears that a light intensity of >400 mW/cm^2 may be required for achieving the optimal dentin bond strength.

INTRODUCTION

Selection of an appropriate adhesive system and a core build-up resin for the restoration of endodontically treated teeth is crucial for the success of treatment. Dual-cure resins have been developed in an attempt to overcome the limitations of chemical-cured and light-cured resins by incorporating a redox initiator system in addition to the photo initiator.[1] It has been reported, however, that dual-cure resins have poorer mechanical properties when the polymerization reaction is limited to a chemical-cure mode alone.[2,3] Although important for resin pastes, proper light irradiation of the adhesive is also required to achieve the optimal dentin bond strength.

Previous studies revealed that the bond strength value was higher when photo-cure bonding agents with sufficient light irradiation time were used for bonding to root canal dentin than when dual-cure systems were used.[4] The apical region of the root canal poses additional difficulties in terms of light curing, which is likely to be the main reason for the lower bond strength in this region.[5] Because the accessibility of light energy passing through a deep and narrow root canal space is restricted, the bond strength gradually decreases from the coronal to the apical surface of the root canal.[6] Therefore, light irradiation of an adhesive seems to be important for core build-up systems to achieve good adhesion to root canal dentin.[7] Because there is little information on the mechanism by which light irradiation of adhesives affects the dentin bond strength of dual-cure direct core build-up systems, determining the influence of light intensity on the dentin bonding characteristics of these resin systems is required.

Wettability of the conditioned adherent surface with adhesives is important for dentin bonding regardless of the mechanism of bonding (ie, chemical, micromechanical interlocking, or a combination).[8] Proper infiltration and polymerization of adhesive in combination with a core build-up resin is required for the success of treatment because bonding to the root canal surface is always challenged by polymerization shrinkage of the core build-up resins.[9,10] The strength of the bond between dentin and the resin composite depends on several factors, including the characteristics of the dentinal substrate and the ability of the adhesives to wet the adherend.[11] Measurements of the contact angles on the adherent surfaces provide information about the surface free energies that relate to the bonding characteristics of the solids.[12] It is expected that analysis of surface free energy will provide more insight into the evolution of the adhesive performance of direct core build-up systems.

This study aimed to examine the influence of the light intensity of the curing unit on the surface free energy of adhesive-treated dentin and the bond strength of dual-cure direct core build-up systems to bovine dentin. The null hypothesis was that the dentin bond strength and polymerization behavior of dual-cure resin core build-up systems are not affected by the light intensity of the curing unit.

METHODS AND MATERIALS

Core Build-up System

Two dual-cure resin core build-up systems, Clearfil DC Core Automix (DC; Kuraray Noritake Dental Inc, Tokyo, Japan) and UniFil Core EM (UC; GC Corp, Tokyo, Japan), were used in combination with the corresponding manufacturer-provided adhesives, Clearfil Bond SE One (Kuraray Noritake Dental Inc) and Self-Etching Bond (GC Corp) (Table 1). A visible-light curing unit (Optilux 400, Demetron/Kerr, Danbury, CT, USA) was connected to a

Table 1: *Core Build-up Systems Tested in This Study*

Core Build-up System (Manufacturer); Code	Adhesive System (Lot No.)	Resin Paste (Lot No.)
Clearfil DC Core Automix One (Kuraray Noritake Dental Inc, Tokyo, Japan); DC	Clearfil Bond SE One (00005A): MDP, Bis-GMA, HEMA, ethanol, water, filler, CQ	Clearfil DC Core Automix (One 00021AA): Bis-GMA, TEGDMA, dimethacrylate, filler, photo/chemical initiator
UniFil Core EM (GC Corp, Tokyo, Japan); UC	Self-Etching Bond (A: 1208011, B: 1208011): 4-MET, ethanol, water methacrylate monomer, photo/chemical initiator	UniFil Core EM (1208011): UDMA, dimethacrylate, fluoroaluminosilicate glass, photo/chemical initiator

Abbreviations: Bis-GMA, 2, 2bis[4-(2-hydroxy-3-methacryloyloxypropoxy)]phenyl; CQ, dl-camphorquinone; 4-MET, 4-methacryloyloxyethyl trimellitate; HEMA, 2-hydroxyethyl methacrylate; MDP, 10-methacryloxydecyl dihydrogen phosphate TEGMDA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate.

variable-voltage transformer (Type S-130-10, Yamabishi Electric Co, Tokyo, Japan). The light intensities used were 100, 200, 400, and 600 mW/cm^2; these values were determined using a dental curing radiometer (Model 100, Demetron/Kerr). The curing unit was placed on a jig to maintain the distance between the light tip end and the specimen surface (2 mm).

Surface Free Energy

A total of 40 mandibular incisors from 2- to 3-year-old cattle were used as substitutes for human teeth.[13] After separating the roots with a low-speed diamond saw (Isomet 1000, Buehler Ltd, Lake Bluff, IL, USA), the pulps were extirpated and the pulp chamber of each tooth was filled with cotton to avoid penetration of the embedding medium. The labial surfaces of the bovine incisors were ground with wet 240-grit silicon carbide (SiC) paper to produce a flat dentin surface (Ecomet 4, Buehler Ltd). Each tooth was then mounted in self-curing acrylic resin (Trey Resin II, Shofu Inc, Kyoto, Japan) to expose the flattened area and placed in tap water to decrease the temperature rise from the exothermic polymerization reaction of the acrylic resin. The final finish of the dentin surface was accomplished by grinding on wet 600-grit SiC paper. After ultrasonic cleaning in distilled water for one minute to eliminate debris, the surfaces were washed with tap water and dried with oil-free compressed air.

For the DC, one drop of Clearfil Bond SE One was applied on the dentin surface for 10 seconds. After blowing with mild air for five seconds, the adhesives were light polymerized for 10 seconds. For the UC, equal amounts of Self-Etching Bond liquids were mixed for five seconds and applied on the dentin for 30 seconds. After blowing strongly with air for 10 seconds, the adhesives were light polymerized for 10 seconds. Light irradiation was performed at intensities of 0 (no irradiation), 200, 400, and 600 mW/cm^2.

The surface free energies of five specimens per group for each adhesive-treated dentin surface were determined by measuring the contact angle on the surface for three test liquids, namely distilled water, 1-bromonaphthalen, and ethylene glycol, each of which has known surface free energy parameters. The Drop Master DM500 apparatus (Kyowa Interface Science, Saitama, Japan) was fitted with a charge-coupled device camera, which allowed automatic measurement of the contact angles (Figure 1).[14]

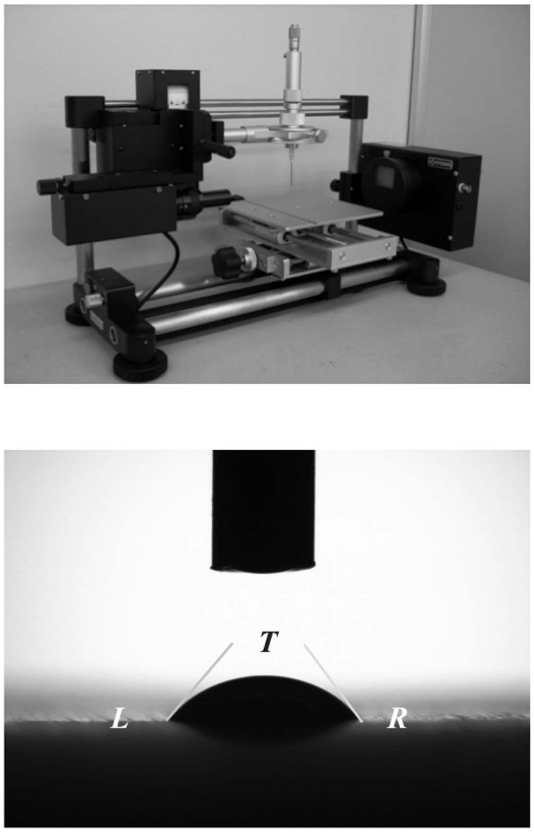

Figure 1. *Drop Master DM500 apparatus (Kyowa Interface Science) fitted with a charge-coupled device camera allowing automatic measurements of the contact angles to be made.*

For each test liquid, the equilibrium contact angle (θ) was measured for five specimens of each adhesive using the sessile-drop method at 23°C ± 1°C. The surface free energy parameters of the solids were then determined on the basis of the fundamental concepts of wetting.[15]

The Young-Dupré equation describes the work of adhesion (W) for a solid (S) and a liquid (L) that are in contact as follows:

$$W_{SL} = \gamma_L + \gamma_S - \gamma_{SL} = \gamma_L(1 + \cos\theta)$$

Here, γ_{SL} is the interfacial free energy between the solid and liquid, γ_L is the surface free energy of the liquid, and γ_S is the surface free energy of the solid.

By extending the Fowkes equation, the γ_{SL} is expressed as follows:

$$\gamma_{SL} = \gamma_L + \gamma_S - 2(\gamma_L^d\gamma_S^d)^{1/2} - 2(\gamma_L^p\gamma_S^p)^{1/2} - 2(\gamma_L^h\gamma_S^h)^{1/2}$$

$$\gamma_L = \gamma_L^d + \gamma_L^p + \gamma_L^h, \gamma_S = \gamma_S^d + \gamma_S^p + \gamma_S^h$$

where γ_L^d, γ_L^p, and γ_L^h are components of the surface free energy (γ) arising from the dispersion force, the polar (permanent and induced) force, and the hydrogen-bonding force, respectively. The θ values were determined for the three test liquids, and the surface energy parameters of the treated dentin surfaces were calculated on the basis of equations using add-on software and the interface measurement and analysis system (FAMAS, Kyowa Interface Science).

Dentin Bond Strength

A total of 80 mandibular incisors from cattle were treated as described in the "Surface Free Energy" section. A piece of double-sided adhesive tape with a 4-mm-diameter hole was firmly attached to define the adhesive area for bonding. A Teflon mold (2-mm high, 4-mm diameter) was used to form and hold the resin pastes to the dentin surface. The dentin surfaces were treated according to the methods described for surface free energy measurements. The auto-mixed pastes were directly inserted into the mold on the adhesive-treated dentin surface, followed by light polymerization for 40 seconds with the same light intensities as those used for the adhesives.

The mold and adhesive tape were removed from the specimen 10 minutes after placement of the resin pastes. Subsequently, the specimens were stored in distilled water maintained at 37°C for 24 hours. The specimens in each group were tested in shear mode using a knife-edge testing apparatus in a universal testing machine (Type 4204, Instron Corp, Canton, MA, USA) at a crosshead speed of 1.0 mm/minute. The shear bond strengths were calculated in megapascals by dividing the peak load at failure by the bond surface area. After testing, the specimens were examined under an optical microscope (SZH-131, Olympus Ltd, Tokyo, Japan) at a magnification of 10× to determine the location of the bond failure. Types of failures were determined on the basis of the predominant percentage of substrate free material as follows: adhesive failure, cohesive failure in composite, and cohesive failure in dentin.

Statistical Analysis

The dentin bond strength data obtained were analyzed using a commercial statistical software package (Sigma Stat, Version 3.1, SPSS Inc, Chicago, IL, USA). Because the data were normally distributed (Kolmogorov-Smirnov test), two-way analysis of variance (ANOVA) was used to analyze the effect of the core build-up system and that of light intensity. Multiple comparisons were then conducted using the Tukey-Kramer test, with the significance level set at 0.05.

Scanning Electron Microscopy

The resin-dentin interface was ultrastructurally observed by scanning electron microscopy (SEM). Bonded specimens from each group (n = 5) were stored in distilled water maintained at 37°C for 24 hours, embedded in self-curing epoxy resin (Epon 812, Nisshin EM, Tokyo, Japan), and stored at 37°C for an additional 12 hours. The embedded specimens were sectioned through the diameter of the composite resin post, and the surfaces of the cut halves were polished with an Ecomet 4/Automet 2 (Buehler Ltd) using SiC papers with a grit size of 600, 1200, and 4000 in succession. The surface was finally polished by a soft cloth using diamond paste (Buehler Ltd) with a grit size of 1.0 μm. All SEM specimens were dehydrated in ascending concentrations of tert-butanol (50% for 20 minutes, 75% for 20 minutes, 95% for 20 minutes, and 100% for two hours) and transferred to a critical-point dryer for 30 minutes. These surfaces were subjected to Argon ion-beam etching (Type EIS-200ER, Elionix Ltd, Tokyo, Japan) for 30 seconds, with the ion beam (accelerating voltage, 1.0 kV; ion current density, 0.4 mA/cm^2) directed perpendicularly to the polished surface. Surfaces were coated in a vacuum evaporator (Quick

Table 2: *Influence of the Light Intensity of the Curing Unit on the Surface Free energy (mJ·m^{-2}) of Adhesive-Treated Dentin**

	Intensity	γ_S^d	γ_S^p	γ_S^h	γ_S
DC	0	41.1 (0.2)	3.1 (0.7)	24.9 (1.7)	69.1 (2.1)
	200	41.1 (0.1)	1.8 (0.5)	16.4 (1.1)	59.3 (2.0)
	400	39.8 (0.3)	0.7 (0.3)	8.9 (0.6)	49.4 (1.7)
	600	39.2 (0.2)	0.7 (0.3)	8.8 (0.6)	48.7 (1.6)
UC	0	41.1 (0.2)	5.6 (0.9)	21.0 (1.6)	67.7 (2.2)
	200	41.1 (0.3)	3.9 (0.9)	17.6 (1.2)	62.6 (2.1)
	400	40.0 (0.4)	1.4 (0.6)	14.9 (1.1)	56.3 (2.0)
	600	39.8 (0.3)	1.0 (0.6)	14.8 (1.1)	55.6 (1.8)

** Values connected by horizontal lines indicate no statistically difference (p>0.05).*

Coater Type SC-701, Sanyu Denshi Inc, Tokyo, Japan) with a thin film of gold and observed by SEM (ERA 8800FE, Elionix Ltd) at an accelerating voltage of 10 kV. All of the specimens were observed under SEM.

RESULTS

The surface free energies and their components for the cured adhesives are shown in Table 2. The total surface free energy ($\gamma_S = \gamma_S^d + \gamma_S^p + \gamma_S^h$) values decreased with an increase in light intensity, and they were significantly lower when adhesives were irradiated with a light intensity of >400 mW/cm^2. For all surfaces, the estimated γ_S^d values remained relatively constant in the range of 39.2 to 41.1 mJ·m^{-2}. Decreases in γ_S^p and γ_S^h values were observed for the specimens that were light irradiated with a stronger light intensity.

The influence of the light intensity of the curing unit on shear bond strengths is shown in Table 3. Two-way ANOVA revealed that the type of core build-up system ($p<0.001$) and the light intensity ($p<0.001$) significantly influence on the bond strength, although there was no significant interaction between the two factors ($p=0.806$). For both core build-up systems, greater bond strengths were achieved when a light intensity of 400 mW/cm^2 or greater was used. Statistically significant differences were observed between the obtained values, except

Table 3: *Influence of the Light Intensity of the Curing Unit on Dentin Bond Strengths (in MPa) of Direct Resin Core Build-up Systems*

	Light Intensity (mW/cm^2)			
	0	**200**	**400**	**600**
DC	12.8 (1.8) a[a]*	15.8 (1.8) b	17.5 (2.7) c	17.8 (3.3) c
Failure mode[b]	0/0/10	1/0/9	7/0/3	8/0/2
UC	10.0 (2.4) d	12.3 (2.0) e	15.2 (1.7) f	15.7 (2.9) f
Failure mode	0/0/10	1/0/9	6/0/4	5/0/5

[a] *Values in parentheses indicate standard deviations.*
[b] *Failure mode: Cohesive failure in resin/cohesive failure in dentin/adhesive failure.*
** Values with the same letter are not significantly different (p>0.05).*

for those obtained at a stronger light intensity of 400 and 600 mW/cm^2 ($p<0.050$). With regard to the failure modes recorded for each group, the predominant failure mode was adhesive failure between dentin and the adhesive resin when a weaker light intensity of 200 mW/cm^2 or less was used. When the resin pastes were irradiated with stronger light intensities, the failure mode changed to cohesive failure.

SEM observations of the resin-dentin interface are shown in Figure 2. The dentin-resin interface of both groups showed excellent adaptation, with the formation of a transitional layer between the adhesive resin and tooth structure. The thickness of the adhesives was lesser for the specimens irradiated with a weaker light intensity of <200 mW/cm^2.

DISCUSSION

The surface free energy of organic substances (γ_S) generally has three components: dispersion (γ_S^d), polar (γ_S^p), and hydrogen (γ_S^h) bonding.[14] The dispersion force (γ_S^d) represents the London interactions between apolar molecules, whereas the polar (nondispersion) force (γ_S^p) represents the electric and metallic interactions in addition to the dipolar interactions. In addition to these two parameters of the γ_S, the hydrogen-bonding force (γ_S^h), which relates to the water and hydroxyl components, was calculated in the current study. Because the hydration of the adherend is of major importance to the wettability behavior related to dentin bonding, the polar interactions, including the dipole and hydrogen-bonding characteristics, should be accurately estimated for their interactions with water. A separate estimation of the dipole (polar) interactions, apart from the hydrogen-bonding interactions, may provide a novel insight into the mechanisms contributing to wettability as well as the bonding characteristics of the adhesives. According to Hata, Kitazaki, and Saito,[16] the Fowkes equation for interfacial free energy can be extended to an interface that includes intermolecular interactions of polar and hydrogen bonding as well as dispersion bonding.

Changes in surface free energy are expressed by the sum of the geometrical means of the components. Contact angle data for the following three types of liquid were used for calculating the surface free energy: purely nonpolar (1-bromonaphthalene), polar (diiodomethane), and hydrogen-bonded (water).[17] The γ_S^d values of the dentin surfaces remained relatively constant (39.2 to 41.1 mJ·m^{-2}) regardless of the adhesives used, and there were no significant differences among groups.[18] The γ_S^p values of all the specimens were relatively low (ranging from 0.7 to 5.6 mJ·m^{-2}) and decreased with increasing light intensity. The γ_S^p value involves the polar interaction, which is often called the nondispersion force, and refers to hydrophilic interactions.[18] Functional monomers are capable of releasing protons and can potentially dissolve the smear layer and interact with the subadjacent intact dentin.[19,20] This finding can be explained by the increase in the chemical reaction with the exposed hydroxyapatite crystals. Although the smear layer is hydrophobic, it holds water within. Moreover, it is porous, so initial partial spreading may have occurred by the infiltration of liquid through the layer.[21] The hydrophilic monomers may form a complex structure with exposed collagen fibrils and partially demineralized dentin containing residual hydroxyapatite. A previous

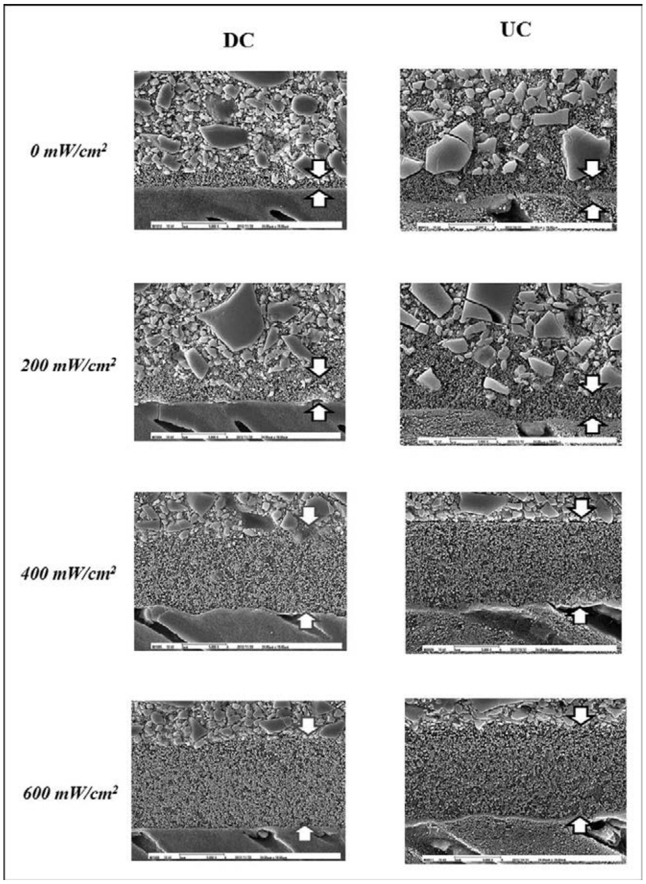

Figure 2. *Representative SEM photomicrographs of the core build-up resin-dentin interface (original magnification, 5000×). Thickness of the adhesive was indicated by the white arrows in each SEM photomicrograph. The dentin-resin interface of both core build-up systems showed excellent adaptation, with the formation of a transitional layer between the adhesive resin and the tooth structure. The thickness of the adhesives was lesser for the specimens irradiated with a weaker light intensity of <200 mW/cm^2.*

study that examined the chemical bonding efficacy of the functional monomers found that it had high potential for chemical bonding to hydroxyapatite and a clinically acceptable application time.[22]

The values for γ_S^h ranged from 21.0 to 24.9 $mJ \cdot m^{-2}$ for the group without irradiation and from 8.8 to 14.8 $mJ \cdot m^{-2}$ for the groups irradiated with a light intensity of 400 mW/cm^2 and greater. Functional monomers have three distinct structural components: functional, spacer, and polymerizable groups. The functional group exhibits hydrophilic properties that enhance the wetting and demineralization of the tooth surface.[23] The spacer group influences the properties of the monomer, including water solubility, and its size determines viscosity, wetting, and penetration behaviors.[24] After light irradiation with stronger light intensities, the conversion of the polymerizable group of functional monomers was enhanced, leading to a more hydrophobic nature of the cured adhesives. The structure of the polymer network will be affected by the monomer composition, initiator, solvent, and light energy.[25] Sufficient light energy may enhance the polymerization reaction and continue the double-bond conversion of resin monomers in the adhesives. Furthermore, the calcium salt of the functional monomer was highly insoluble and was able to resist ultrasonic rinsing with ethanol. According to the adhesion-decalcification concept,[26] the less soluble the calcium salt of the acidic molecule, the more intense and stable the molecular adhesion to a hydroxyapatite-based substrate. The long carbonyl chain of 10-methacryloyloxydecyl dihydrogen phosphate renders this functional monomer relatively hydrophobic.[24] Because the dentin surface was covered by such a hydrophobic layer after light irradiation with a stronger light intensity, relatively lower γ_S^h values were obtained with the DC. Moreover, incomplete polymerization of adhesive would lead to residual water within the adhesive.[27] Irradiation of the adhesive with a weaker light intensity may impair optimal formation of the cross-linking network of hydrophobic monomers due to the lower concentration of hydrophobic cross-linking monomers and incomplete polymerization of the bonding resin; this would lead to lower γ_S^h values of the adhesives irradiated with a weaker light intensity.

When the core build-up system was cured with a greater light intensity, the dentin bond strength values were higher. On the other hand, lower bond strengths were achieved when the core build-up systems were polymerized with a light intensity of 200 mW/cm^2 or less. These data may suggest inferior physical properties due to low double-bond conversion of resin monomers.[28] From SEM observation of the dentin-resin interface, the thickness of the adhesive was much less in the group irradiated with a weaker light intensity. This indicates insufficient polymerization of the adhesive due to the lower light energy. Even if complete penetration into the demineralized dentin can be achieved, the degree of conversion of resin monomers may be impacted by the insufficient light energy. For the same reason, the mechanical properties of the material filled in the coronal region were better than those of the material filled in the apical region.[4] Therefore, the properties of dual-cure core build-up resins may be different in different regions of the post cavity due to the decreased light energy in the deeper regions of the cavity; this may also affect regional bond strengths.

The adhesives of the core build-up systems were light irradiated directly during the bonding procedures and indirectly through the core build-up resins. Light intensity of the indirect light irradiation of adhesive layer may be affected by the shade and thickness of core build-up resins. It was reported that the indirect light irradiation through the resin composite contributes to higher dentin bond strength of self-etch adhesives.[29] From the results of the present study, the type of core build-up system and the light intensity significantly influenced the bond strength, although there was no significant interaction between the two factors. The polymerization process of the bonding resin is influenced by the type and concentration of photo-initiators and catalysts used in the adhesives as well as light intensity. The photo-initiation system and light intensity of the curing unit might have a complex effect on dentin bond strength of the adhesives. Furthermore, one must consider that this *in vitro* study used flat dentin for the adherent surface, which might be different than the canal wall dentin because contraction stress induced by polymerization of core build-up resin causes reduction of bond strength in clinical situations.[30]

The adhesive systems used in this study are categorized as self-etching systems containing acidic functional monomer. When core build-up resin is irradiated, the hydrophobic resin matrix can copolymerize with the oxygen-inhibited layer produced on cured adhesives.[31] Irradiation of the self-etch adhesives with a weaker light intensity retards the polymerization process, leading to the creation of a thicker oxygen-inhibited layer. Presumably, there would be remnants of acidic functional monomer with water and solvent in the oxygen-inhibited layer of self-etch adhesives. It is possible that the polymerization ability of the core build-up resin is affected by the acidic moieties because tertiary amines in the resin paste may be neutralized by the acidic functional monomers in the uncured adhesive.[32] The adhesive functional monomers affect the polymerization of CQ/amine catalysts, resulting in poor interaction with the resin paste.[33] It has been reported that the thickness of the oxygen-inhibited layer of the adhesive may be changed by the total light energy supplied from a curing unit. When the total light energy supply increases, the degree of conversion increases and the thickness of the oxygen-inhibited layer decreases.[34] Incompatibility between the adhesives and the core build-up resin does not occur under stronger light intensity conditions. However, improper polymerization occurs when the resin pastes are polymerized with weaker light intensities, including 0 and 200 mW/cm^2, which may lead to adverse interaction between the nucleophilic tertiary amine and acidic functional monomers as previously stated.

CONCLUSIONS

With regard to the dual-cure resin direct core build-up systems evaluated in this study, polymerization with a higher light intensity resulted in lower surface free energy of the adhesive-treated dentin surfaces because the conversion of the polymerizable group of functional monomers was enhanced. Sufficient light energy may enhance the polymerization reaction and continue the double-bond conversion of resin monomers in the adhesives, leading to higher bond strength to dentin. If the core build-up resin was polymerized with a light intensity of 200 mW/cm^2 or less, a stronger hydrogen-bonding force was observed in the applied adhesives, leading to inferior bonding performance perhaps being due to the presence of water. The data suggest that the dentin bond strength and polymerization behavior of dual-cure, direct core build-up systems are still affected by the light intensity of the curing unit.

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Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Effect of Hydrogen Peroxide Concentration on Enamel Color and Microhardness

AB Borges • RF Zanatta • ACSM Barros
LC Silva • CR Pucci • CRG Torres

Clinical Relevance

This study showed that 35% hydrogen peroxide gel exhibited higher whitening potential compared with 20% gel, without intensifying the adverse effects on enamel surface microhardness.

SUMMARY

Objectives: The aim of this study was to investigate the effect of hydrogen peroxide gels with different concentrations (20%, 25%, 30%, and 35%) on enamel Knoop microhardness (KNH) as well as on changes in dental color (C).

Methods: Cylindrical specimens of enamel/dentin (3-mm diameter and 2-mm thickness) were obtained from bovine incisors and randomly divided into six groups (n=20), according to the concentration of the whitening gel (20%, 25%, 30%, 35%, control, thickener). After polishing, initial values of KNH_0 and color measurement, assessed by spectrophotometry using the CIE L*a*b* system, were taken from the enamel surface. The gels were applied on the enamel surface for 30 minutes, and immediate values of KNH_i were taken. After seven days of being stored in artificial saliva, new measures of KNH_7 and color (L_7^* a_7^* b_7^*, for calculating ΔE, ΔL, and Δb) were made. Data were submitted to statistical analysis of variance, followed by Tukey test ($p<0.05$).

Results: Differences in gel concentration and time did not influence the microhardness ($p=0.54$ and $p=0.29$, respectively). In relation

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to color changes, ΔE data showed that the 35% gel presented a higher color alteration than the 20% gel did (p=0.006).

Conclusion: Bleaching with 35% hydrogen peroxide gel was more effective than with the 20% gel, without promoting significant adverse effects on enamel surface microhardness.

INTRODUCTION

The pursuit of an esthetic smile has stimulated the search for effective treatments and alternatives to increase its attractiveness. Tooth whitening is a highly desirable esthetic treatment, since it is conservative and can lead to satisfactory results for changing dental color.[1] Hydrogen peroxide is an important agent used in dental bleaching that is capable of penetrating tooth structures, releasing free radicals, and oxidizing chromophore molecules, by means of redox processes.[2] The penetration of these oxidative agents in dental structures breaks these chromophore molecules into less complex molecules, giving a brighter aspect to the tooth.[3]

Dental bleaching procedures can be performed in a dental office, with total control of the dentist, or by the patient at home, with professional supervision. Although both techniques are shown to be effective,[4] in the in-office technique, higher concentrations of the hydrogen peroxide gel are usually used to reduce the clinical treatment time to 30 to 60 minutes.[5] The concentration of hydrogen peroxide in the whitening gel has an inverse correlation with the application time needed for achieving satisfactory outcomes;[6] thus, for faster results, with fewer applications, higher concentrations of hydrogen peroxide are required.[7]

However, there are some concerns regarding potential adverse effects that can happen to dental tissues after dental whitening. The results are controversial, but some authors claim that alterations in enamel surface morphology can happen,[8-10] as well as significant changes in microhardness values after bleaching.[8,11,12] In addition, Bistey and others[13] observed that significant structural alterations, with important loss of phosphate ions, occurred in the enamel surface when high concentrations of hydrogen peroxide (greater than 20%) were used. Furthermore, slightly erosive effects in bleached enamel were also described as promoted by the whitening agent.[10]

Therefore, investigations concerning bleaching efficacy of different hydrogen peroxide concentrations and the possible adverse effects on enamel are important to determine ideal protocols for better outcomes and less damage to tooth structure using the in-office technique. The aim of this study was to evaluate the color and microhardness of the enamel submitted to whitening treatments with hydrogen peroxide gels in different concentrations (20%, 25%, 30%, and 35%), immediately after application and after seven days. The null hypothesis tested was that higher concentrations of hydrogen peroxide do not improve the whitening effect and do not change enamel microhardness.

METHODS AND MATERIALS

Sample Preparation

Freshly extracted, undamaged, and intact bovine incisors were selected and stored in 0.1% thymol solution until required. One hundred enamel-dentin specimens 3 mm in diameter and 2 mm in height (1 mm of enamel and 1 mm of dentin) were prepared from the buccal surface of the tooth using a diamond trephine mill (Dentoflex, São Paulo, SP, Brazil).[14] Enamel and dentin thickness were standardized, ground flat, and polished with sequential water-cooled silicon carbide paper discs (1200-, 2400-, and 4000-grit; Fepa-P, Struers, Ballerup, Denmark). The enamel surfaces were verified with a stereomicroscope (Carl Zeiss, Stemi 2000-20×), and the surfaces presenting cracks and imperfections were discarded. The specimens were immersed in deionized water, placed in an ultrasonic bath for 10 minutes (Ultrasonic Cleaner, Odontobras, Ribeirão Preto, Brazil), and then stored in distilled water for rehydration. Specimens were randomly divided into six groups (n=20), according to the concentration of the hydrogen peroxide whitening gel: control (distilled water), thickener (gel without peroxide), 20%, 25%, 30%, and 35%.

Color Measurement

Prior to each bleaching treatment, the initial color of all specimens was taken. The baseline color coordinates were assessed in standard conditions using a reflectance spectrophotometer (CM-2600d Konica Minolta, Osaka, Japan).[5] The device was adjusted to use the D65 light source with 100% ultraviolet and specular reflection included. The observer angle was set at 2°, and the device was adjusted to a small reading area (SAV) with a total area of 3 mm^2. The spectrophotometer was adjusted for three consecutive measures, which were later averaged. The results of the color measurement were quantified in terms of the L*, a*, b* coordinate values established by the Commission Internatio-

Table 1: *Microhardness Mean Values (± SD) Obtained for Tested Groups*

Group	Initial	After Bleaching	After Seven Days
Control	347.41 (±23.16)	342.51 (±22.49)	348.03 (±15.73)
Thickener	351.91 (±21.68)	345.29 (±20.86)	341.08 (±16.43)
20%	352.75 (±25.39)	348.09 (±37.35)	330.38 (±42.18)
25%	353.17 (±20.68)	356.46 (±30.56)	344.81 (±27.18)
30%	353.68 (±22.53)	351.48 (±33.30)	352.62 (±40.00)
35%	353.11 (±22.99)	346.93 (±33.82)	355.64 (±45.38)

nale de l'Eclariage (CIE), in which the L* axis represents the degree of lightness within a sample and ranges from 0 (black) to 100 (white). The a* plane represents the degree of green/red color, and the b* plane represents the degree of blue/yellow color.[5,15]

The measurement of color change after the bleaching procedures was made by calculating the variation of L* (ΔL), a* (Δa), and b* (Δb). The total color change (ΔE) was calculated according to the following formula[15]:

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{½}$$

Microhardness Measurement

The initial surface microhardness (KNH_0) of all specimens was obtained before the bleaching procedures using a microhardness tester (FM-700, Future-Tech, Tokyo, Japan), with a Knoop indenter, under 25 g load for 10 seconds. Three indentations were made in each sample, 100 μm apart, and the average was calculated for KNH_0.

Bleaching Procedures

The whitening gels used in this study were experimental and manipulated in our laboratory, following the protocol described previously,[6] resulting from the mixture of two parts: the first was a solution of 50% hydrogen peroxide containing an acrylic thickener, which in an acidic environment is a white solution (solution A). The second part consists of an aqueous solution containing an alkaline substance (solution B). They were manually mixed immediately before application in a 3:1 proportion by volume of peroxide base gel and thickener, respectively. Immediately after mixing, the pH of all gels was calculated using a pH meter (Digimed DM-20, Digicrom Analítica Ltda, São Paulo, Brazil), with an electrode (Digimed DME-CV8) calibrated with buffer solutions of pH 4.00 and 6.86. The pH of thickener gel and 20%, 25%, 30%, and 35% gels was, respectively, 6.07, 5.66, 5.71, 5.70, and 5.36.

The products were applied over controlled conditions of temperature (25°C) and humidity (50%). A 1 mm layer of whitening gel was applied over the enamel surface of each specimen for 10 minutes and repeated three times, totaling 30 minutes of application. This protocol of application was chosen to simulate the clinical application defined for many gels available for clinical use. An aspiration cannula was used to remove the gel in between each application, simulating clinical bleaching treatment. After application, the specimens were washed with deionized water and submitted to an immediate measurement of microhardness (KNH_i) following the same specifications previously described for initial measurements. The specimens were then stored in artificial saliva, manipulated according to Gohring and others,[16] during seven days, with daily changes. After the storage period, new measures of microhardness (KNH_7) and color (L_7* a_7* b_7*, for calculation of ΔE, ΔL and Δb) were performed.

Statistical Analysis

After analyzing for normal distribution, data were submitted to analysis of variance (ANOVA), where the microhardness (KNH) was analyzed by repeated-measures ANOVA (with time the repeated variable) and color data (ΔE)—initial and after seven days of bleaching—submitted to one-way ANOVA (with gel pH the variable) and a post-hoc Tukey test ($p<0.05$) to compare the differences among groups.

RESULTS

Microhardness mean values are shown in Table 1. The repeated-measures ANOVA showed no significant difference among groups ($p=0.60$), or for the time factor ($p=0.15$), or the interaction groups × time ($p=0.45$). For the color data (Table 2), the one-way ANOVA test showed significant differences among groups for the values of ΔL ($p=0.0001$), Δb ($p=0.0001$), and ΔE ($p=0.0001$).

Table 2: *Mean (±SD) Color Change Parameters for Each Group*

Group[a]	ΔL	Δb	ΔE
Control	0.48 (±1.96)a	0.18 (±0.82)a	2.00 (±0.83)a
Thickener	−0.64 (2.04±)ab	0.42 (±0.81)a	2.09 (±0.98)ab
20%	1.55 (±1.07)bc	−2.19 (±1.73)b	3.15 (±1.25)bc
25%	2.25 (±1.03)c	−2.93 (±1.70)bc	4.03 (±1.36)cd
30%	2.21 (±0.88)c	−3.42 (±0.81)c	4.16 (±1.00)cd
35%	2.69 (±1.17)c	−3.59 (±1.28)c	4.61 (±1.52)d

[a] Different letters indicated significant differences among groups ($p<0.05$).

DISCUSSION

The null hypothesis tested was denied for color alteration, since a higher concentration of hydrogen peroxide in the bleaching gel proved to be more efficient in tooth whitening.

The gel concentration did not cause significant changes in the enamel surface microhardness, either immediately after application or after seven days. Indeed, previous data showed that the use of hydrogen peroxide does not alter enamel histomorphology or microhardness.[17] While some studies have observed demineralization of enamel submitted to bleaching procedures,[8,18,19] these structural modifications have been assigned mostly to the gel pH,[19-21] usually at less than 5.2. In fact, Sulieman and others[17] affirmed that studies reporting adverse effects on bleached enamel and/or dentin do not reflect the bleach itself; instead, they reflect the pH of the formulation used. Thus, neutral gels are recommended for tooth bleaching with the purpose of reducing deleterious effects on tooth enamel.[21] The gels tested in the present study have a pH between 5.3 and 5.7, and showed no significant changes for the time in contact with the enamel, which may have been insufficient to promote enough mineral modification to impact the enamel microhardness. Furthermore, Bistey and others[13] reported that, besides hydrogen peroxide concentration, structural changes of the enamel surface are also time dependent, with considerable changes happening at greater than 60 minutes of exposure to peroxide. The time of exposure used in this study was 30 minutes, as recommended by many bleaching gels, which might be insufficient to promote enamel demineralization. Also, previous *in situ* bleaching studies reported a decrease in enamel microhardness on placebo groups treated with thickener agents, such as carbopol and poloxamer.[22,23] The action mechanism by which these agents cause this reduction is still unknown, but it is speculated that they have an acidic nature. In the present study, we included a group using a gel (pH 6.07) with the same basic composition as the tested gels but without the peroxide, so we could verify that the thickener was unable to promote mineral dissolution by itself.

Almost all morphological studies evaluating the side effects of bleaching in dental tissues have been conducted *in vitro*, because it is difficult to perform microhardness, roughness and microscopic analysis *in vivo*. Amaral and others[24] evaluated the calcium and phosphorus concentration in human enamel *in vivo* and found no differences between in-office (35% and 38%) and home use (10% and 20%) bleaching gels. In addition, Metz and others[25] found no differences in enamel microhardness *in vivo* using teeth extracted for orthodontic reasons.

Regarding the color changes, the strength of the carbon bonds present in the chromophore molecules is inversely proportional to the dental color, meaning that molecules presenting carbonic rings in their structure absorb more light than linear chains with unsaturated double-bond molecules, and these, on the other hand, absorb more light than saturated linear chains without double bonds. Therefore, the higher the light absorption by complex molecules, the lower the reflection, giving the sensation of a darker tooth, requiring a higher acting time of the whitening gel or a higher concentration of the hydrogen peroxide.[26] Thus, according to Kawamoto and Tsujimoto,[27] higher concentrations of hydrogen peroxide solutions present larger amounts of free radicals, increasing the whitening potential. Indeed, in this study, the higher concentration of hydrogen peroxide (35%) promoted a better whitening effect on the enamel after seven days of the procedure, compared with the 20% concentration. Since the hydrogen peroxide that penetrates enamel prisms can be active for several days until being completely neutralized,[28] the color measurement was made after this period to allow enamel rehydration and color stability.[29]

The color change analysis is a complex issue, since the most important parameter of color reading is controversial. Color differences evidenced by the spectrophotometer might not necessarily be clinically relevant; however, the use of a spectrophotometer in this study is justified by the improvement in the standardization of shade assessment, allowing accuracy and reproducible results for color measurements, compared with the human eye, which presents differences in the color perception.[30,31] According with Dietschi and others,[15] when analyzing the three color dimensions of the CIE L*a*b* system separately, the L* values are important as they determine the lightness by quantifying the black-white color, while a* and b* values describe chroma and are less useful. However, Gerlach and others[28] evaluated the subjective response of individuals submitted to whitening procedures and concluded that the change from yellow to blue (decrease of b* value) is of primary importance in the perception of patients submitted to bleaching procedures, and their satisfaction is associated more strongly with variations in the b* coordinate than with a* and L*. In addition, ΔE describes the global color change, including all three color dimensions of the CIE L*a*b* system, and can also be used to compare the efficacy of different bleaching agents.[15] However, clinical color alteration impression is not well established in the literature. The threshold of color alteration perception has been reported as $\Delta E=1$, but the threshold of color alteration acceptability has been stated as $\Delta E=3.7$.[32]

Karpinia and others[33] observed in an *in vitro* study that bleached tooth presented a significant decrease of the Δb (reduction in the yellow color) and an increase of ΔL (brightness). These parameters were also verified in this study, and specimens bleached with higher-concentrated hydrogen peroxide gel (35%) showed greater values of ΔL and ΔE and lower values of Δb, indicating a better whitening performance of this gel. This better whitening performance of higher hydrogen peroxide concentrated gels was also verified in *in vitro*[5] and *in vivo*[7] studies, which showed that bleaching gel with 35% hydrogen peroxide promoted a better whitening effect compared with 10% carbamide peroxide gel and 20% hydrogen peroxide gel, with similar treatment times, respectively. Based on these findings, it can be suggested that the use of whitening gels with 35% hydrogen peroxide can be indicated for patients who desire a faster whitening effect with minor influence on enamel surface properties.

Nevertheless, as it was conducted *in vitro*, this study presents some limitations, especially regarding the absence of pulp tissue, making it impossible to predict the side effects of high-concentration gels on tooth sensitivity and pulp cells,[34] as well as the absence of pulp pressure, which can interfere in the penetration of the gel in vital teeth.[35]

CONCLUSION

Enamel microhardness was not influenced by different concentrations of hydrogen peroxide gels. The 35% hydrogen peroxide gel exhibited higher whitening potential than the 20% gel, without intensifying the side effects on the enamel surface property tested.

Conflict of Interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Transdentinal Cell Photobiomodulation Using Different Wavelengths

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Clinical Relevance

Determining the optimal irradiation parameters of odontoblast-like cell stimulation using dentinal barrier as a function of the wavelength is the first step toward establishing the ideal window for biostimulation of pulp tissue previously injured by caries lesion progression and cavity preparation.

SUMMARY

Objective: The aim of this study was to investigate the effects of transdentinal irradiation with different light-emitting diode (LED) parameters on odontoblast-like cells (MDPC-23).

Methods and Materials: Human dentin discs (0.2 mm thick) were obtained, and cells were seeded on their pulp surfaces with complete culture medium (Dulbecco modified Eagle medium). Discs were irradiated from the occlusal surfaces with LED at different wavelengths (450, 630, and 840 nm) and energy densities (0, 4, and 25 J/cm^2). Cell viability (methyltetrazolium assay), alkaline phosphatase activity (ALP), total protein synthesis (TP), and cell morphology (scanning electron microscopy) were evaluated. Gene expression of collagen type I (Col-I) was analyzed by quantitative polymerase chain reaction (PCR). Data were

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analyzed by the Mann-Whitney test with a 5% significance level.

Results: Higher cell viability (21.8%) occurred when the cells were irradiated with 630 nm LED at 25 J/cm^2. Concerning TP, no statistically significant difference was observed between irradiated and control groups. A significant increase in ALP activity was observed for all tested LED parameters, except for 450 nm at 4 J/cm^2. Quantitative PCR showed a higher expression of Col-I by the cells subjected to infrared LED irradiation at 4 J/cm^2. More attached cells were observed on dentin discs subjected to irradiation at 25 J/cm^2 than at 4 J/cm^2.

Conclusion: The infrared LED irradiation at an energy density of 4 J/cm^2 and red LED at an energy density of 25 J/cm^2 were the most effective parameters for transdentinal photobiomodulation of cultured odontoblast-like cells.

INTRODUCTION

Inflammatory pulp reaction is commonly observed subjacent to active decay. The intensity of tissue inflammation may increase during mechanical cavity preparation and following cavity restoration with dental materials with nonbiocompatible components. About and others[1] reported that the sum of damages experienced by the pulp tissue can result in pain and exacerbate local inflammatory reactions. Therefore, several products and techniques have been proposed to prevent or relieve pulp sensitivity and biostimulate the healing of this specific connective tissue. Phototherapy has appeared as a promising treatment for this purpose.[2]

Recent studies have demonstrated that light-emitting diode (LED) irradiation is capable of stimulating cells to synthesize collagen-rich matrix and proteins that play a role in its mineralization.[3-5] Additionally, several *in vitro* experiments with LED at different wavelengths have demonstrated a significant increase in the proliferation of fibroblasts, osteoblasts, muscle cells in rats, human epithelial cells, and mesenchymal stem cells.[4-7] Other positive effects caused by light, such as reduced dentin sensitivity, formation of mineralized tissue stimulus, improvement in rheumatoid arthritis, and mucositis healing, have also been reported.[8-12]

Despite these interesting scientific data, little is known about the transdentinal effects of LED irradiation on pulp cells. In current studies, it was demonstrated that the power density that reaches cells is much lower than the one applied to dentin, mainly because of light scattering.[13,14] For 0.2 mm, specifically, the mean of power loss in dentin discs is approximately 40.0%.[14] Some recent studies have shown that phototherapy promotes biomodulation when applied directly to cells with an odontoblast phenotype, increasing the synthesis and expression of dentin matrix proteins.[15] Therefore, it could be speculated that, in clinical situations, the transdentinal LED biomodulation of odontoblasts subjacent to the dental cavity may cause deposition of tertiary dentin, protecting the pulp tissue against further assaults from different sources.[16] It is known that transdentinal irradiation causes an effective decrease of energy density.[13,14] Although the energy density applied on the external surface is known, the energy density that actually reaches the cell layer is much lower due to light scattering. One purpose of this study was to determine that, even with this loss, LED irradiation is capable of causing biostimulatory effect on odontoblast-like cells.

Since there is no previous information about the possibility of transdentinal stimulation of odontoblasts by LED irradiation, the aim of this study was to evaluate whether specific LED parameters at different wavelengths are capable of diffusing through a 0.2-mm-thick dentin barrier to biostimulate cultured odontoblast-like MDPC-23 cells.

METHODS AND MATERIALS

LED Devices and Irradiation Parameters

Irradiation was performed with three devices (LEDTables) containing 24 diodes with wavelengths at 450, 630, or 840 nm. The InGaN diodes (indium, gallium, and nitride) were individually positioned in the LEDTables in such a way that each could homogeneously irradiate the cells attached to the bottom of a well in a 24-well plate. During cell irradiation, the LED device was applied in noncontact mode and perpendicular to the bottom of the well. The distance between the dentin disc and the LED device tip was 2.0 cm.

The energy densities of 4 and 25 J/cm^2 used in this investigation were selected based on previous studies in which the authors irradiated different cell types.[5,15,17] The irradiance emitted by the LEDs was 88 mW/cm^2, and the power loss caused by the plate and the dentin disc was considered, resulting in irradiation times of 1 minute and 20 seconds (4 J/cm^2) and 8 minutes and 40 seconds (25 J/cm^2). For all groups, cells were maintained in contact with phosphate-buffered saline (PBS) for the irradiation procedure, as previously described.[18]

Temperature Monitoring

PBS temperature variations were evaluated by means of a multimeter (38XR; Metermam, Everett, WA, USA) and a calibrated thermistor (38XR, Metermam) placed at the bottom of the well. This preliminary analysis was performed because PBS heating may cause cell damage.

Obtaining Dentin Discs

This study was approved by the Research Ethics Committee (Protocol 26/09) of the Araraquara School of Dentistry, UNESP, University Estadual Paulista, Brazil. One hundred and eighty-two human dentin discs from sound human molars were obtained, selected by stereomicroscopy as previously described,[13,14] and reduced to 8-mm diameter. The dentin surfaces were rinsed with 0.5 M ethylenediaminetetraacetic acid (pH 7.2) according to a previous study.[19] The discs were then washed with sterile deionized water for 60 seconds and were subjected to measurement of transdentinal LED light transmission.

Measurement of Transdentinal LED Light Attenuation

This test was performed to determine the light attenuation through the disc structure and provide a homogeneous distribution of discs among groups, according to the power loss values of each one. The protocol of transdentinal power measurement was described in detail in a previous study.[13]

MDPC-23 Cell Culture

The MDPC-23 cells were cultivated in Dulbecco modified Eagle medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA), 100 IU/mL of penicillin, 100 μg/mL of streptomycin, and 2 mmol/L of glutamine (Gibco). The cells were maintained in a humidified incubator with 5% CO_2 and 95% air at 37°C (Isotemp, Fisher Scientific, Bellefonte, PA, USA).

Experimental Conditions

After distribution into the experimental and control groups, the dentin discs were packaged and sterilized by ethylene oxide[19] and, finally, adapted to metal devices designed for this study (Figure 1). The devices and silicon O-rings were autoclaved for 15 minutes at 120°C and 1 kgf of pressure. Each device containing the dentin disc allowed for LED irradiation of the occlusal surface of the disc (facing down), while the pulpal surface on which the cells were seeded remained in contact with the culture medium. The MDPC-23 cells were seeded (3×10^4 cells/disc) on the pulp surfaces of the discs and incubated for 48 hours at 37°C and 5% CO_2.

After this period, the culture medium was replaced by a new culture medium supplemented with 2% fetal bovine serum (FBS),[20,21] and the cells were incubated for an additional 24 hours. Immediately before irradiation, the culture medium was replaced by sterile buffered saline solution (PBS) at room temperature.[18] The 24-well plates were then placed on the LEDTables (Figure 1) for specific periods, according to the energy density. A single LED irradiation was performed to simulate a clinical situation in which a deep cavity is available for restoration. Cells were irradiated in a dark room; thus, the LED irradiation was the only light source that the cells were exposed to.

After irradiation, the PBS was aspirated, and 1 mL of fresh culture medium containing 10% FBS was added to each well. Following incubation for 72 hours, the cell viability (methyltetrazolium [MTT] assay), alkaline phosphatase (ALP) activity, total protein (TP) synthesis, and cell morphology (scanning electron microscopy [SEM]) were evaluated. The expression of collagen type I (Col-I) was analyzed by real-time polymerase chain reaction (RT-PCR). In the control group, the same cell manipulation procedures were performed, but the diodes were not activated.

Cell Viability (MTT assay)

Cell viability (n=8) was evaluated using the MTT assay (Sigma-Aldrich), which determines the activity of SDH enzyme produced by mitochondria in cells. The dentin discs were carefully removed from the devices and individually placed in wells of 24-well plates. The MTT assay was performed as described in previous studies.[15,21]

TP Production and ALP Activity

Eight samples were selected to evaluate TP production and ALP activity.

TP Production—The production of TP was measured for each experimental and control group according to the protocol described in a previous study by the Lowry method.[15]

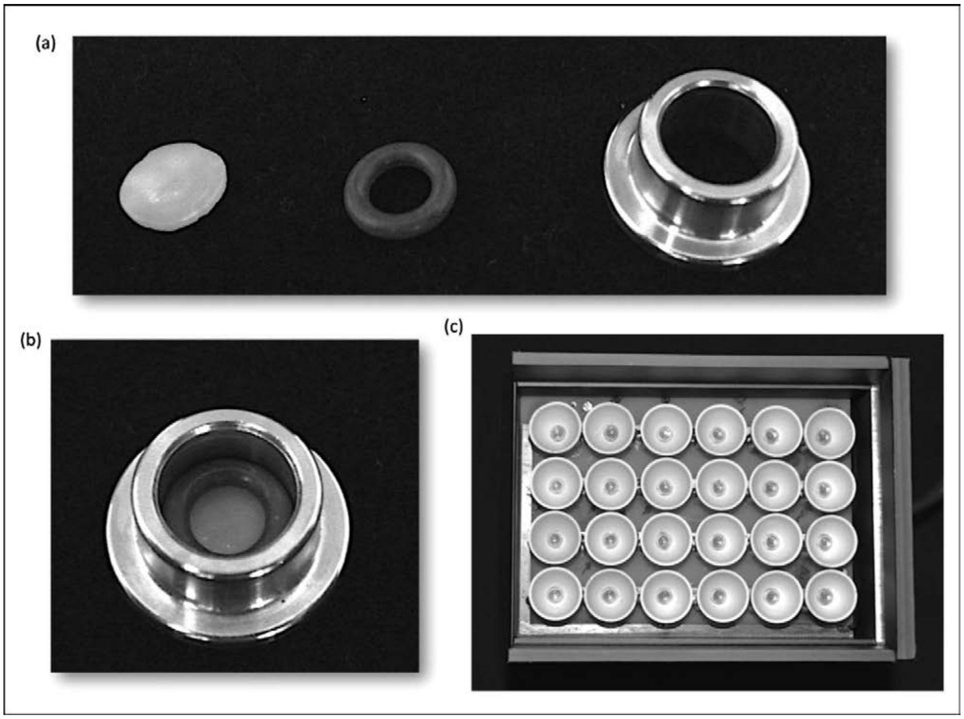

Figure 1. *Illustration of the dentin disc/device set and irradiation apparatus (LEDTable). (a): Dentin disc, silicon O-ring, and the irradiation apparatus, separately. (b): The dentin disc/device set. (c): Top view of LEDTable with 24 diodes and their collimators used to irradiate the dentin discs.*

ALP Activity—ALP activity was evaluated according to the protocol of the Alkaline Phosphatase Kit-colorimetric endpoint assay (Labtest Diagnóstico SA, Lagoa Santa, MG, Brazil). This assay utilizes thymolphthalein monophosphate, a substrate of phosphoric acid ester. ALP hydrolyzes the thymolphthalein monophosphate, releasing thymolphthalein. The enzymatic activity was measured as previously described.[15]

Analysis of Cell Morphology by SEM

For each experimental and control group, samples were prepared for cell morphology analysis by SEM (JEOL-JMS-T33A Scanning Microscope; JEOL USA Inc, Peabody, MA, USA). The laboratory protocol employed in this study is well established and has been widely used.[15,19]

Col-I Expression—RT-PCR

RNA extraction was performed by the Trizol method, which was detailed by Basso and others.[22] The cDNA was obtained by means of the High Capacity cDNA Reverse Transcriptions Kit (Applied Biosystems, Foster City, CA, USA) as previously described.

After cDNA synthesis, the effect of LED irradiation was assessed on the expression of Col-I with β-actin as the endogenous control. For each of these genes, specific primers were designed from the mRNA sequence (Table 1).

The reactions were prepared with standardized reagents for RT-PCR SYBR® Green PCR Master Mix (Applied Biosystems) in addition to sets of primers for each gene. Fluorescence readings were performed by Step One Plus (Applied Biosystems) in each amplification cycle and were subsequently analyzed by Step One Software 2.1 (Applied Biosystems). All reactions were subjected to the same analysis conditions and normalized by the signal from the passive reference dye ROX to correct for fluctuations in readings due to changes in volume and evaporation during the reaction. Individual results expressed in CT values were transferred to spreadsheets and grouped according to the experimental groups, normalized according to expression of the endogenous gene selected (β-actin). Then the

Table 1: *Primer Sequences and Applications for Mus musculus Used in This Work*

Primer Sequences	Gene
S: 5′-AGC CAT GTA CGT AGC CAT CC-3′	βAct
AS: 5′-CT CTC AGC TGT GGT GGT GAA-3′	
S: 5′-TGA GGT CCA GGA GGT CCA-3′	Col-I
AS: 5′-AAC TTT GCT TCC CAG ATG TCC-3′	

concentrations of target gene mRNA were evaluated statistically.

Statistical Analysis

The data set for each variable—cell viability, TP production, ALP activity, and Col-I expression—were evaluated concerning their distribution. In compliance with the requirements for the selection of parametric tests, nonparametric Kruskal-Wallis tests were complemented by the Mann-Whitney test, set at a predetermined significance of 5%.

RESULTS

Temperature Monitoring

The 630-nm wavelength (red LED) yielded no temperature increase during 10-minute irradiation, which was the maximum time used for cell irradiation. The 450-nm (blue LED) and 840-nm (infrared) wavelengths caused an increase of only 2°C after 8 minutes of irradiation. Thus, the temperature rise did not cause damage to cells; temperature increase of up to 3.4°C does not cause detrimental effects on MDPC-23 cells.[23]

Cell Viability (MTT Assay), TP, ALP, and Col-I Expression

The data for cell viability, ALP, TP, and collagen type-I expression, according to the energy densities and wavelengths used in this study, are shown in Figure 2. When the LED energy densities were compared for cell viability, it was observed that for the 450-nm wavelength (blue LED), there was no statistical difference between the irradiated groups and the control, which was considered with 100% cell viability ($p>0.05$). For the 630-nm wavelength (red LED), the energy density of 25 J/cm^2 increased the cell viability by 21.8% ($p<0.05$). For the 840-nm wavelength (infrared LED), energy densities of 4 and 25 J/cm^2 reduced the cell viability by 18.6% and 29.1%, respectively; all of them were statistically different from the control group ($p<0.05$). Concerning the LED wavelengths at the energy density of 4 J/cm^2, 450 nm (blue light) caused statistically higher cell viability compared to 840 nm ($p<0.05$). For energy density of 25 J/cm^2, the cells irradiated with red LED (630 nm) presented greater viability compared to the infrared LED (840 nm; $p<0.05$).

Concerning ALP, it was observed that the wavelengths used for all irradiated groups showed higher values of ALP activity when compared to the control group, except for the blue LED at 4 J/cm^2 ($p>0.05$). For the wavelength of 450 nm (blue LED), the group irradiated with 25 J/cm^2 provided statistically better results than the control group ($p<0.05$), with an increase of 113% in ALP activity. For the wavelength of 630 nm (red LED), groups irradiated with 4 and 25 J/cm^2 also showed statistically greater values when compared with the control group ($p<0.05$), with an increase of 46.7% and 81.7% in ALP activity, respectively. For 840 nm wavelength (infrared LED), groups irradiated with 4 and 25 J/cm^2 also had higher levels of ALP ($p<0.05$), and the increase in ALP activity compared to the control group was 220% and 121%, respectively. When the wavelengths were compared, it was observed that there was no difference among the groups for 25 J/cm^2 ($p>0.05$). However, when the irradiation was performed with 4 J/cm^2, increased ALP activity was observed for the wavelength of 840 nm ($p<0.05$).

For TP, regarding the energy densities used for this *in vitro* study, it was observed that there was no statistical difference between the irradiated and control groups for all wavelengths ($p>0.05$). But when the wavelengths were compared, there was a statistically significant difference between the 630-nm wavelength (red LED) and the 840-nm wavelength (infrared LED) only for 4 J/cm^2, and the wavelength representing the red region spectra showed the best results ($p<0.05$).

Finally, for Col-I expression, comparing the energy densities of 4 and 25 J/cm^2, it was possible to determine that the 450-nm irradiation resulted in a decrease of 64% and 56% in the Col-I expression, respectively. For the red LED, the group of 4 J/cm^2 was not statistically different from the control group ($p>0.05$). However, the energy density of 25 J/cm^2 showed a decrease of 40% in the expression of Col-I, and this difference was statistically significant when compared to the control group ($p<0.05$). For the infrared LED, an energy density of 4 J/cm^2 caused a 168% increase in the Col-I expression when compared with the control group ($p<0.05$). Moreover, the energy density of 25 J/cm^2 did not differ statistically from the control group ($p>0.05$). When the wavelengths were compared, it was observed that for 4 J/cm^2, there was a statistically significant difference among all evaluated wavelengths ($p<0.05$). However, the 840-nm wavelength (infrared) showed the best results for the expression of Col-I, followed by 630 nm (red) and 450 nm (blue). At 25 J/cm^2 energy density, the 840-nm wavelength showed significantly higher values compared to 450 and 630 nm ($p<0.05$).

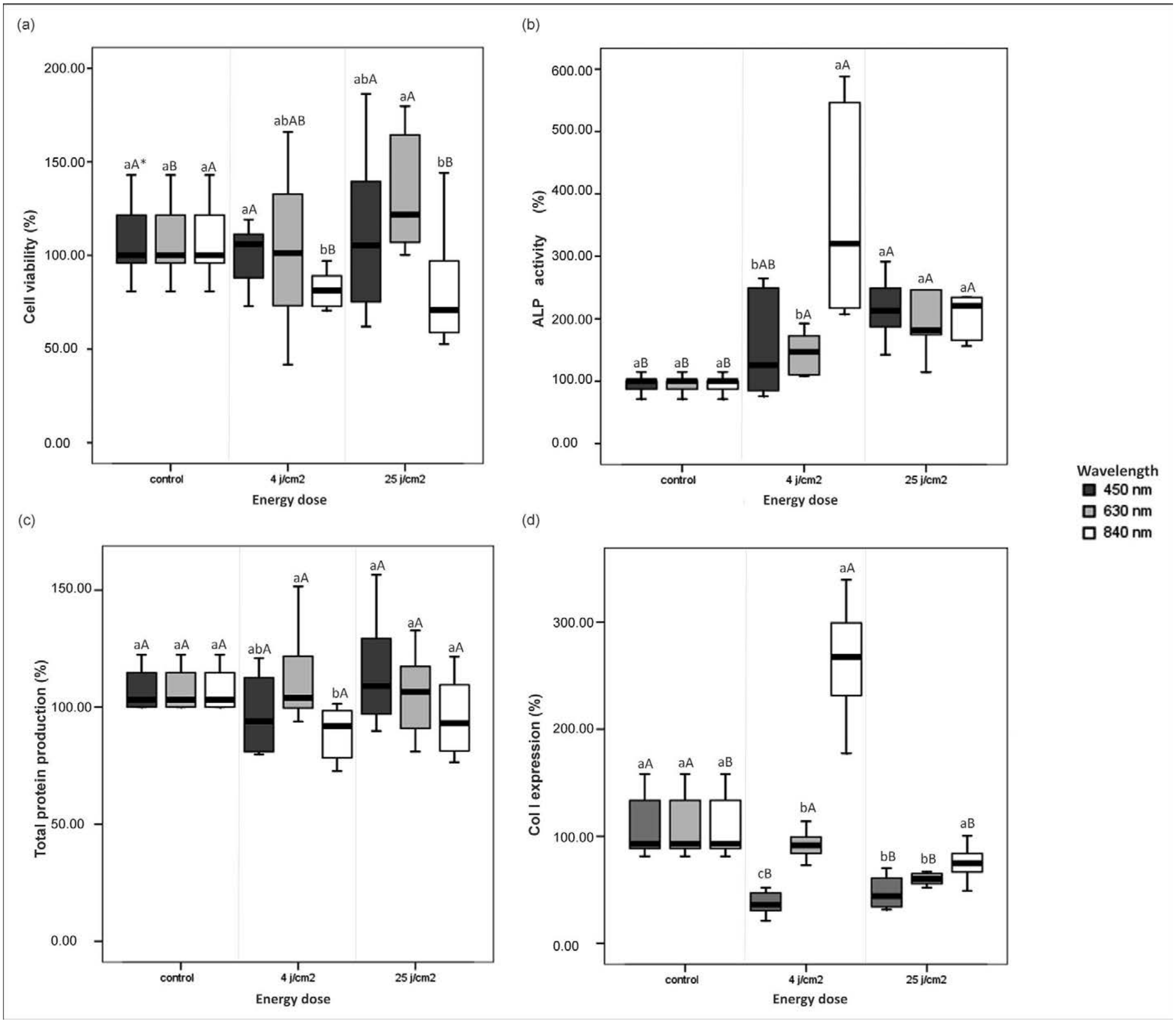

Figure 2. *Box plot indicating data for cell viability, total protein(TP), alkaline phosphatase (ALP) activity, and collagen type I (Col-I) expression. (a): Cell viability (%) detected by the methyltetrazolium (MTT) assay according to energy densities and wavelengths. (b): ALP activity (%) according to the energy densities and wavelength. (c): TP production (%) according to the energy densities and wavelength. (d): Col-I expression (%) according to the energy densities and wavelength (n=8). *Uppercase letters allow for comparison among energy densities, and lowercase letters allow for comparison among wavelengths. Same letters indicate no statistically significant difference (Mann-Whitney, p>0.05).*

Cell Morphology—SEM

Cells subjected to LED irradiation presented number and morphology similar to that of control group cells. More attached cells were observed on dentin discs subjected to irradiation at 25 J/cm^2 than at 4 J/cm^2 (Figure 3).

DISCUSSION

LED therapy has been used in different areas of human health, especially in dermatology[24-26] and neurology.[17] Additionally, this type of light has also been employed for muscle analgesia, anti-inflammatory effect,[7,27,28] and regeneration of injured tissues.[29,30] Specifically, in dentistry, LED has been evaluated as an alternative adjuvant therapy for treatment of mucositis,[11] dentin hypersensitivity,[10,31] and pulp cell stimulation.[32]

Tate and others[9] irradiated sound molars of rats with low-power laser and observed an intense formation of mineralized tissue within 30 days after

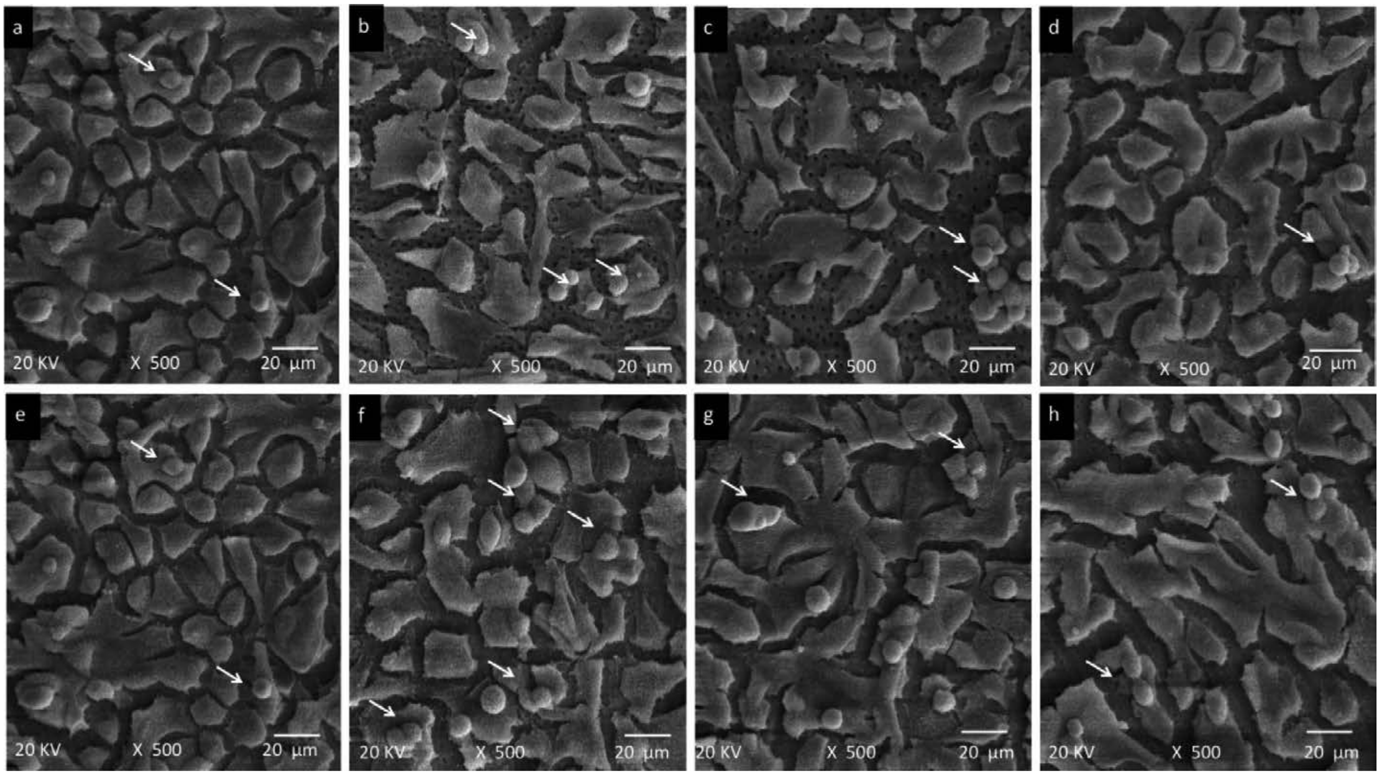

Figure 3. *Panel of SEM micrographs representative of cell morphology for each group. (a-d): Control group, 450 nm, 630 nm, and 840 nm LED, respectively, for 4 J/cm^2. (e-h): Control group, 450 nm, 630 nm, and 840 nm LED, respectively, for 25 J/cm^2. Arrows indicate mitosis. More attached cells were observed on dentin discs subjected to irradiation at 25 J/cm^2 than at 4 J/cm^2. Magnification 500×.*

procedure. However, the light irradiation of dentin-pulp complex able to promote biostimulation of human pulp cells previously subjected to an aggressive stimulus is not well known. To cause any effect on the pulp tissue, light must be transmitted through the hard dental tissues to reach the pulp cells with sufficient energy density to induce biostimulation. Despite the scarce basic research data concerning the optimal parameters of light application for different therapeutic activities, the clinical effects of light have already been assessed and even used as adjuvant therapy.[33] Therefore, this study evaluated the effect of transdentinal LED irradiation on odontoblast-like MDPC-23 cells seeded on dentin discs, using three different wavelengths, for which some positive cellular effects were described in the literature.[5,6,12,34]

In the present study, a significant increase in cell metabolism was observed in the group in which cells were subjected to irradiation with red LED at an energy density of 25 J/cm^2. In contrast, cells irradiated with infrared LED showed decreased metabolism after 72 hours of irradiation. Vinck and others[35] irradiated fibroblasts with red and infrared LED and observed increased cell metabolism 24 hours after irradiation and decreased cell metabolism 72 hours after irradiation. The authors suggested that this reduction in fibroblast metabolism at 72 hours postirradiation may be due to the occurrence of cell confluence after a long incubation, leading to contact-cell inhibition. This inhibitory phenomenon may also explain the negative effects obtained in the present study when the cells were irradiated with infrared wavelengths. The SEM analysis showed that, no matter the energy densities and wavelengths evaluated in this study, the morphology of MDPC-23 cells did not change. Thus, it may be suggested that the infrared LED, at energy densities of 4 and 25 J/cm^2, affected negatively the cell metabolism without causing detectable changes in cell morphology. Other studies have also evaluated the effect of light on cell metabolism, and generally the diverse responses found after irradiation were increased metabolism after 24[35,36] and 72 hours[31,37] and metabolism similar to the control group.[4,38] Importantly, the responses of irradiated cells were always dependent on the type of cell culture and irradiation parameters employed. Thus, cells that showed increased metabolism 24 hours after the last irradiation were from a fibroblastic lineage, seeded at a density of 7 × 10^4 cells/cm^2, incubated for 24 hours, and subjected to three daily irradiations.[35,36] Particularly, this irradiation therapy used by Vinck and others)[36] does not match the protocol tested in the present study since the purpose of this study was to simulate a clinical situation in which a very deep dental cavity needs to be restored. Then, for economic, practical, and time reasons, the clinician has the possibility of irradiating the cavity floor one time only before filling the cavity. Also, since the cells internally lining the dentin tissue are odontoblasts, MDPC-23 cells were used in this study because they have the odontoblast phenotype and have been widely employed in various studies of light biomodulation.[15,21,31,38]

In addition to cell metabolism, ALP activity was evaluated. An increased expression of this enzyme by irradiated cells may indicate an interesting improvement in pulp tissue healing. However, previous studies showed that depending on the irradiation parameters used, different responses may occur in the ALP activity.[31,38] This cell behavior has been confirmed in the present study, where there was an increase in the activity of this protein for almost all irradiated groups. Thus, considering the positive results of ALP activity and correlating it with increased cell metabolism, particularly for the red LED at an energy density of 25 J/cm^2, it may be speculated that this parameter could be studied more deeply in future research both *in vitro* and *in vivo*. Moreover, other studies have shown an increase in ALP expression by mesenchymal cells irradiated with red LED.[4,5] Therefore, besides biomodulation of odontoblast-like cells, LED-specific parameters may also act positively on mesenchymal stem cells, which, in the case of injury by external factors, such as caries, heating, trauma, and so on, can effectively participate in the repair of the pulp-dentin complex.

Regarding Col-I expression, it was observed that irradiation with the infrared wavelength, at an energy density of 4 J/cm^2, increased the expression of this gene by 167% compared with that of the control group. Previous studies that evaluated the expression of Col-I by laser and LED phototherapy, in the red and infrared spectra, reported either similar behavior of irradiated groups compared with the control group[21] or increased expression of this gene after irradiation.[3,4] This result demonstrates that the use of this wavelength in transdentinal irradiation may be effective in forming nonmineralized matrix.

In general, the red light stood out from the other wavelengths, as did the blue light, which caused the lowest cell stimulation. These data can be explained, at least in part, by the occurrence of high scattering when lower wavelengths, like blue light, are applied to tissues, decreasing the energy density that reaches the local cells.[12,14] It is known that infrared light has the lowest scattering; however, it shows lower absorption by cells when compared with the red light.[12,39] Thus, it may be suggested that simultaneous effects of scattering and absorption by cultured pulp cells subjected to transdentinal LED irradiation occurred in the present investigation. It is known that wavelengths above 500 nm are better absorbed by cytochrome *c* oxidase, resulting in a greater synthesis of adenosine triphosphate by the cell with consequent increase of energy by oxidative phosphorylation.[40] On the other hand, flavins are small water-soluble molecules known to initiate free radical reactions when excited by light at wavelengths below 500 nm.[41] This information may explain, at least partially, the different cell responses found in the present study.

The results of this study underscore the importance of determining the optimal parameters for cellular biomodulation by LED phototherapy. For this study, wavelength and energy densities were factors that interfered with the cellular responses to transdentinal irradiation with LEDs, and all three wavelengths were able to cross the dentin barrier and cause some stimulus on pulp cells. Further *in vivo* studies are required to elucidate the effects of LED on the pulp-dentin complex as well as to determine whether LED irradiation may interact with different cells at the same time to trigger distinct pathways of biomodulation and tissue repair.

CONCLUSION

Based on the data obtained in this *in vitro* study, it can be concluded that infrared LED irradiation (840 nm) at an energy density of 4 J/cm^2 and red LED (630 nm) at an energy density of 25 J/cm^2 were the most effective parameters for transdentinal photobiomodulation of cultured odontoblast-like cells.

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Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Evaluation of Tensile Retention of Y-TZP Crowns Cemented on Resin Composite Cores: Effect of the Cement and Y-TZP Surface Conditioning

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Clinical Relevance

Adhesive cementation using adhesive resin cements promotes higher crown retention when compared with self-adhesive, resin-modified ionomer, and zinc phosphate cements. Conditioning with tribosilicatization and application of a thin low-fusing glass porcelain layer plus silanization on the intaglio surface of an yttria-stabilized polycrystalline tetragonal zirconia crown seems to improve crown retention.

SUMMARY

This study evaluated the effect of the cement type (adhesive resin, self-adhesive, glass ionomer, and zinc phosphate) on the retention of crowns made of yttria-stabilized polycrystalline tetragonal zirconia (Y-TZP). Therefore, 108 freshly extracted molars were embedded in acrylic resin, perpendicular to their long

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axis, and prepared for full crowns: the crown preparations were removed and reconstructed using composite resin plus fiber posts with dimensions identical to the prepared dentin. The preparations were impressed using addition silicone, and Y-TZP copings were produced, which presented a special setup for the tensile testing. Cementation was performed with two adhesive resin cements (Multilink Automix, Ivoclar-Vivadent; RelyX ARC, 3M ESPE, St Paul, MN, USA), one self-adhesive resin cement (RelyX U100, 3M ESPE), one glass ionomer based cement (RelyX Luting, 3M ESPE), and one zinc phosphate cement (Cimento de Zinco, SS White, Rio de Janeiro, Brazil). For the resin cement groups, the inner surfaces of the crowns were subjected to three surface treatments: cleaning with isopropyl alcohol, tribochemical silica coating, or application of a thin low-fusing glass porcelain layer plus silanization. After 24 hours, all groups were subjected to thermocycling (6000 cycles) and included in a special device for tensile testing in a universal testing machine to test the retention of the infrastructure. After testing, the failure modes of all samples were analyzed under a stereomicroscope. The Kruskal-Wallis test showed that the surface treatment and cement type (α=0.05) affected the tensile retention results. The Multilink cement presented the highest tensile retention values, but that result was not statistically different from RelyX ARC. The surface treatment was statistically relevant only for the Multilink cement. The cement choice was shown to be more important than the crown surface treatment for cementation of a Y-TZP crown to a composite resin substrate.

INTRODUCTION

Zirconia crowns have superior flexural strength (> 1000 MPa)[1] and fracture toughness (> 9-10 MPam$^{1/2}$)[2] than those of other ceramics due to the presence of tetragonal crystals stabilized by yttrium oxide in the material microstructure. By means of thermal, mechanical, or chemical stimulation, these tetragonal grains transform into a monoclinic arrangement, leading to a significant increase in grain volume, generating localized compressive stresses that hinder both slow and fast crack propagation.[3] The outstanding mechanical behavior of yttria-stabilized polycrystalline tetragonal zirconia (Y-TZP) makes it an excellent candidate material to be used as the infrastructure of both all-ceramic crowns veneered with porcelain and monolithic prostheses.[4]

However, the physical and chemical characteristics of Y-TZP do not favor bonding to current resin cements.[5,6] Although it has been reported by Casucci and others[7] and Blatz and others[8] that resin-based luting agents are the most appropriate materials for the purposes of marginal seal, retention, and fracture resistance, the use of surface pretreatments to improve bonding to the luting agent is mandatory in this case. Different surface treatments have been proposed to modify the zirconia surface and promote mechanical and/or chemical adhesion.[9-13] According to Inokoshi and others,[14] the combination of mechanical and chemical pretreatments can be recommended to promote a stable bond to zirconia.

Sandblasting with aluminum oxide particles coated by silica, followed by the application of silane (silanization),[15,16] has been widely used to increase surface roughness (micromechanical retention) and to establish chemical bonds with resin cements.[17,18] An alternative method is the application of a thin layer of glassy porcelain (rich in silica), followed by etching with hydrofluoric acid and silanization.[12,19-23] According to Ntala and others,[21] that surface treatment increases the capacity of the Y-TZP surface to establish both chemical and micromechanical interactions with resin composites.

Another important factor to be considered when using all-ceramic restorations is the substrate on which the crown will be cemented. When the dentin remaining is insufficient, it is recommended to use an intraradicular post and a composite core buildup to support the crown. In this clinical situation, the use of prefabricated fiber posts associated with a composite core will have a positive impact on the final esthetic result of a cemented zirconia crown.[24] However, it is important to keep in mind that the adhesion process of resin cements to dentin is significantly different than that observed for composite resins. A resin composite core has very few unreacted methacrylate groups at the surface when the cementation procedure takes place. This fact reduces the potential for bonding to a new resinous material, such as the resin cement.[25] In order to improve the adhesion forces, it is necessary to modify the composite core surface to improve chemical bonding to resin cements. An example of a surface treatment for the resin core is the use of self-etching adhesives, which have been proven to promote high bond strength values between aged and new resin due to an efficient wettability provided by the self-etching systems.[26]

Therefore, it can be concluded that it is necessary to assess not only the bond strength of the resin cements to dentin but also the interface between the resin cement and the composite resin core. Thus, the objective of this study was to compare different treatments of the inner surface of frameworks made of Y-TZP and different types of cements in terms of crown retention (tensile forces) in preparations made mostly of resin composite. The hypotheses were that 1) the different adhesive cements would lead to similar crown retention values; 2) adhesive resin cements would promote higher retention values than the self-adhesive, glass ionomer, and zinc phosphate cements; and 3) the Y-TZP intaglio surface treatment would improve retention regardless of the cement used.

METHODS AND MATERIALS

In order to determine the sample size, a sample calculation was made based on two other articles.[27,28] Considering a statistical power of 80%, mean standard deviation of 1.7, and a detectable difference of 2.3 MPa, the sample size was established as n = 12, for a total of 108 teeth.

The teeth were donated by the Human Teeth Bank of the Federal University of Santa Maria and stored in distilled water (4°C) until needed. The 108 teeth were numbered and assigned randomly into nine testing groups (Table 1), using a computer program (www.randomizer.org).

Embedding the Teeth

The coronal part of the tooth was glued to an adapted surveyor to keep the long axis of the tooth perpendicular to the ground (horizontal plane) when embedding each root into the acrylic resin. Self-cured acrylic resin (Dencrilay, Dencril, Caieiras, SP, Brazil) was prepared and poured into the matrix. Then the tooth was inserted into the resin, up to 3 mm below the cemento-enamel junction.

Tooth Preparation

The occlusal surfaces of all teeth were cut with a diamond blade mounted on a cutting machine (Isomet 1000, Buehler, Lake Bluff, IL, USA), 4 mm above the cemento-enamel junction. Conical trunk diamond burs (KG 3139 and KG 3139FF, KG Sorensen, Cotia, Brazil) were mounted in a high-speed hand piece and fixed to a modified optic microscope to obtain reductions as parallel as possible to the long axis of the tooth. Thus, the axial walls were reduced at depths of 1.5 mm (similar to the bur diameter), and a standard angle of convergence was created. The height of the preparation was 4 mm.

Composite Core

1) A vinyl polysiloxane impression (Elite Light Body-normal set, Zhermack, Badia Polesine, Italy) of each dental preparation was performed. Then master dies were produced, and a silicone matrix was fabricated on each master die. Thus, the future composite core reconstruction had the same characteristics as the full crown preparation for each tooth.
2) All of the dental preparations were removed by cutting with a diamond blade (Isomet 1000).
3) For the post cementation, the greatest root canal of each molar received intracanal preparation with a custom #2 drill of the glass fiber post system (White Post DC, FGM, Joinville, Brazil). Afterward, the coronal and root dentin received an etch-and-rinse single-bottle adhesive system (Ambar, FGM). The dentin was etched with 37% phosphoric acid, rinsed with water for 20 seconds, and dried with absorbent papers. The adhesive agent was then applied according to the manufacturer's instructions and light-cured for 20 seconds (Radii-Cal, SDI, Australia). The posts received silane application (Prosil, FGM) and were cemented with a dual-cure resin cement (Allcem, FGM). The core was built up with a composite resin (Opallis, Joinville, Brazil), using the silicone matrix previously made. All preparations were finished with a fine conical trunk diamond bur (3139FF, KG Sorensen) under low rotation. The specimens were stored in water (37°C) for 24 hours.

Crown Manufacture

The preparations of the specimens were molded with polyvinylsiloxane (Elite Light Body-normal set, Zhermack). Afterward, master dies (CAM-BASE type 4, Dentona, Dortmund, Germany) were obtained and taken to the CEREC MC XL IN LAB for the crown manufacture using Software Inlab 3.60. The copings were designed with retentive features on the occlusal surface for subsequent tensile testing, and milling was performed using VITA In-Ceram 2000 YZ CUBES (VITA Zahnfabrik, Bad Säckingen, Germany) and sintered in a Zircomat

Table 1: *Experimental Design*

Groups (n=12)	Cement Type	Composition	Ceramic Surface Treatment Type
MultC	Self-cured, Hema based resin cement (Multilink)[a]	Dimethacrylate, HEMA, phosphonic acid, methacrylate monomers and barium glass, ytterbium trifluoride, spheroid mixed oxide fillers	Sinalization
MultS			Silicatization[b] + silanization
MultV			Vitrification[c] + silanization
RelC	Dual-cured, Bis-GMA based resin cement (RelyX ARC)[d]	Bisphenol-A-diglycidylether, dimethacrylate (BisGMA) and triethylene glycol dimethacrylate (TEGDMA) polymer, zirconia/silica filler	Silanization
RelS			Silicatization + silanization
RelV			Vitrification + silanization
Ion	Resin-modified glass ionomer cement (RelyX Luting)[e]	Fluoroaluminosilicate (FAS) glass, methacrylated polycarboxylic acid, proprietary reducing agent, bisGMA, HEMA, opacifying agent, potassium persulfate, zirconia silica filler	—
Self	Self adhesive cement (RelyX U100)[f]	Glass powder, methacrylated phosphoric acid esters, triethylene glycol, dimethacrylate (TEG-DMA), silane, treated silica, sodium persulfate	—
Zinc	Zinc phophate cement (Cimento de Zinco)[g]	Zinc oxide powder, magnesium oxide, dyes, phosphoric acid, aluminum hydroxide	—

[a] *The tooth and core surfaces were pretreated with the self-etching and self-curing primers system of the cement. The two primer liquids Multilink Primer A and B were mixed and scrubbed on the core for 30 seconds. Dispersed excess with blown air until the mobile liquid film was no longer visible. Pastes A and B of the Multilink Automix cement were squeezed from the dispenser syringe and the mix applied into the crown. The material excess was removed immediately with a microbrush.*

[b] *The inner surfaces were air-abraded with 30 μm aluminum oxide particles coated by silica oxide (Cojet Sand, 3M ESPE, St. Paul, MN, USA). The sandblasting was performed with the aid of a suitable device[22] and a constant pressure of 2.8 bars at a distance of 15 mm from the occlusal infrastructure region, with circular movements, for 20 seconds.*

[c] *Application of a thin layer of low-fusing porcelain glaze (VITA AKZENT, Vita Zanhfabrik, Bad Säckingen, Germany) with the aid of a brush. The infrastructure was subjected to a sintering cycle as recommended by the manufacturer. Afterward, the surface was etched with 10% hydrofluoric acid for 1 minute, washed with water, and air-dried. Subsequently, the copings were cleaned again by an ultrasound device (5 minutes in distilled H_2O).*

[d] *The tooth and composite core surfaces were etched with 37% phosphoric acid, rinsed with water for 5 seconds, and dried with absorbent papers. The Single Bond total-etch single bottle adhesive system (Single Bond, 3M ESPE, St. Paul, MN, USA) was applied according to manufacturer's instructions and photocured for 20 seconds (Radii-cal). The two pastes of the RelyX ARC cement were squeezed from the dispenser syringe and mixed and applied into the crown. The material excess was removed immediately with a microbrush and photocured for 20 seconds from each side.*

[e] *The two pastes of the RelyX Luting cement were squeezed from the dispenser syringe and mixed and applied into the crown. The material excess was removed immediately with a microbrush and photocured for 20 seconds from each side.*

[f] *The two pastes of the RelyX U100 cement (3M ESPE, St. Paul, MN, USA) were squeezed from the dispenser syringe and mixed and applied into the crown. The material excess was removed immediately with a microbrush and photocured for 20 seconds from each side.*

[g] *The powder and the liquid of the zinc phosphate cement (Cimento de Zinco - SS White, Rio de Janeiro, Brazil) were mixed and applied into the crown. Material excess was removed immediately with a microbrush.*

furnace (VITA Zahnfabrik) as recommended by the manufacturer.

Framework Cementation

Before Y-TZP framework cementation, the inner surface of Y-TZP copings of the groups Multilink and RelyX ARC were treated using three different methods, according to Table 1.

After the Y-TZP surfaces were conditioned, the surfaces were silanized with a silane agent (ESPE-Sil, 3M ESPE, St. Paul, MN, USA) that was applied for 60 seconds and then thoroughly air-dried. All of the infrastructures were cemented using a device that exercised a force of 750*g* over the assembled tooth/Y-TZP infrastructure.

Thermocycling

After cementation, all specimens were stored in distilled water at 37°C for 24 hours and then submitted to thermocycling (6000 cycles) between 5°C and 55°C, according to Ernst and others[27] and Palacios and others.[28]

Tensile Test

Before tensile testing, part of the cemented infrastructure was embedded in acrylic resin (Dencrilay, Dencril, SP, Brazil) until the resin covered the retention form on the crown. This procedure was performed following the same axis as used with root embedding with the aid of an adapted surveyor.

For testing, the lower base of the assembly was fixed on a universal testing machine (DL-2000, Emic, Pinhais, PR, Brazil), and the upper base was connected to a mobile device that contained a load cell of 1000 N, and the tensile strength test was performed until fracture with a speed of 0.5 mm/min (Figure 1).

Failure Analysis

The fractured interfacial surfaces of the tested specimens were analyzed under a stereomicroscope

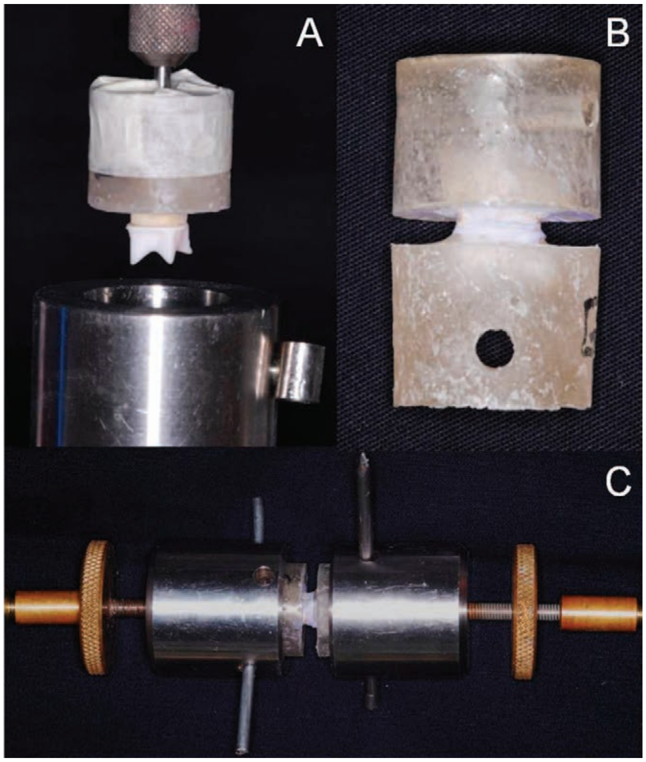

Figure 1. *Construction of the specimen for tensile testing. (A): The embedding of the crown into the specific matrix for tensile test. (B): The root and crown embedding with a hole in the acrylic resin. (C): The tensile test device with the specimen inside.*

(SteREO Discovery V12, Carl Zeiss, Gottingen, Germany). The failure mode was classified as: over 50% of cement in crown; over 50% of cement in substrate; and catastrophic failure (post debonding, root fracture and removal of the root from the acrylic resin).

Micromorphological Analysis

Three Y-TZP discs (10 mm in diameter, 3 mm in thickness) were treated with tribochemical silica coating, vitrification, and no treatment, exactly as if conditioning of the inner surfaces of the framework. The micromorphological changes were inspected using scanning electron microscopy (SEM).

Statistical Analysis

The tensile retention data were submitted to Kruskal-Wallis and Dunn multiple comparison tests (α=0.05). A statistical analysis was performed among the groups that were cemented with Multilink and RelyX ARC in order to compare only adhesive resin cements.

The data of the groups MultC and RelC were subject to the Dunn multiple comparison test to verify the effect of the cement on the crown without surface treatment. The same was performed for the other surface treatments. The data of the groups MultC, MultS, and MultV were subject to the Dunn multiple comparison test to verify the effect of the surface treatment on the crown cemented with Multilink cement. The same was performed for the RelyX ARC cement.

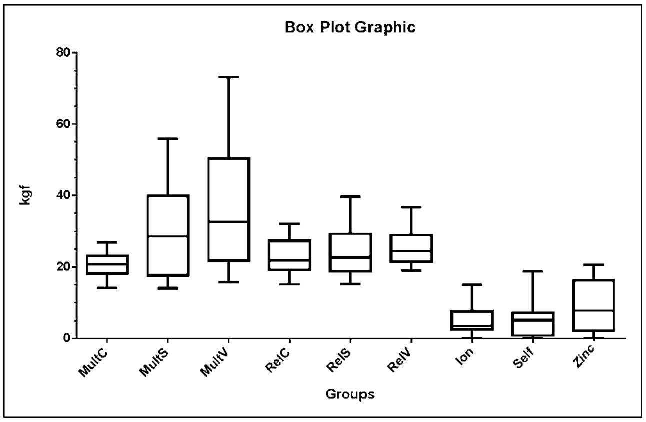

Figure 2. *Box-plot graph of all groups, where the upper and lower vertical lines represent the highest and lowest retention values, respectively. The upper and lower lines of the box represent 75th and 25th percentiles, respectively. The horizontal line represents the median.*

Another statistical analysis was performed among all groups without the zirconia surface treatment. The data of the groups MultC, RelC, Ion, Self, and Zinc were subject to the Dunn multiple comparison test to compare the cement types without the zirconia surface treatment.

RESULTS

The median, maximum, minimum, and first and third quartiles (Q1 and Q3) of all groups are shown in the box plot (Figure 2). The data are shown as mean and standard deviation for better comparison with others studies. The comparisons of the groups cemented with the adhesive resin cements are shown in Table 2. The vitrification method showed significantly higher retention values when compared to the control group (no treatment) only for the groups cemented with Multilink. However, the vitrification and silicatization surface treatments were statistically similar for the Multilink cement. The factor "cement type" was not statistically significant.

Table 3 shows the comparisons between cement groups without conditioning of the framework inner surfaces. Bis-GMA- and HEMA-based resin cements showed the highest retention values. The other cements showed similar retention values.

Table 2: *Mean (±Standard Deviation) of the Tensile Retention Data (kgf) for Different Resin Cement Groups*[a]

Cements	Surface Treatment		
	Control	Silicatization	Vitrification
Multilink	20.5 ± (3.5) Aa	29.9 ± (13.1) ABa	38,8 ± (19.6) Ba
RelyX ARC	22.8 ± (5.3) Aa	25.2 ± (7.8) Aa	25.9 ± (5.3) Aa

[a] *Different capital letters indicate a significant difference (*p*<0.05) between surface treatment types maintaining the same cement (line). Different lowercase letters indicate a significant difference (*p*<0.05) between the cement types maintaining the same surface treatment (column).*

The failure analysis indicated that most of the groups had high percentages of cement remaining over the composite core preparation after the tensile testing, except for crowns cemented with Multilink (Figure 3), since most failures for those crowns were catastrophic. For the crowns cemented with a resin-modified glass ionomer (RelyX Luting), all specimens showed a higher percentage of cement on the crown side. Representative images for the failure analysis can be seen in Figure 4.

Representative SEM images of the conditioned Y-TZP surfaces are shown in Figure 5. Relevant changes were observed after the different surface treatments (Figure 5B,C) when compared to the untreated surface (Figure 5A). Pits and microretentions caused by selective etching with hydrofluoric acid can be seen on the glazed Y-TZP surface (Figure 5C).

DISCUSSION

The retention values obtained for both the chemically activated dual-cure resin cement (Multlink) and the BIS-GMA-based cement were not statistically different from each other. Thus, the first hypothesis of this study was accepted. Multilink Automix contains HEMA molecules, which possess a molecule that contains one hydroxyl radical, increasing the stability of the monomer under moist and acidic conditions.[29] Additionally, Multilink is chemically activated, which minimizes polymerization shrinkage due to the slower polymerization reaction.

Table 3: *Mean (± Standard Deviation) of the Tensile Retention Data (kgf) for Different Resin Cement Groups*[a]

Cements	Median
MultC	20.5 ± (3.5) A
RelC	22.8 ± (5.3) A
Ion	5.4 ± (5.1) B
Self	4.9 ± (4.2) B
Zinc	8.6 ± (7.5) B

[a] *Different letters indicate a significant difference (*p*<0.05) among cement types.*

It is important to note, though, that all three groups cemented with Multilink showed higher percentages of catastrophic failures, especially for group MultS. It may be concluded that the posts debonded from the intracanal dentin before the crown debonded from the preparation, indicating that the bond strength between the post and the dentin substrate was not as high as the one obtained between the cement and dentin. If the post had not debonded from the root canal, the retention values of these groups might have been higher. This observation is in line with the results obtained from Palacios and others,[28] who stated that the tensile retention values of some of the experimental groups were influenced by the cohesive failure of the zirconia coping and the teeth, thereby underestimating the real retention values. In the present investigation, specimens cemented with Multilink showed significantly more catastrophic failures than those cemented with RelyX ARC. Thus, it might be inferred that the Multilink cement could promote greater retention values if the posts had not debonded. According to Luthy and others,[5] RelyX ARC is a conventional dual-cure resin cement (based on BIS-GMA) that does not have any functional monomer in its composition, keeping it from developing any kind of chemical bonding to the crown or the core.

When considering the factor "surface treatment," it was significant only for the Multilink cement, and

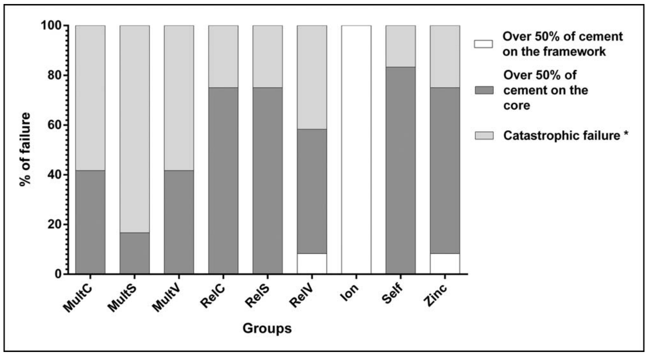

Figure 3. *Percentages of types of failure for each cement. Catastrophic failure*:post debonding.*

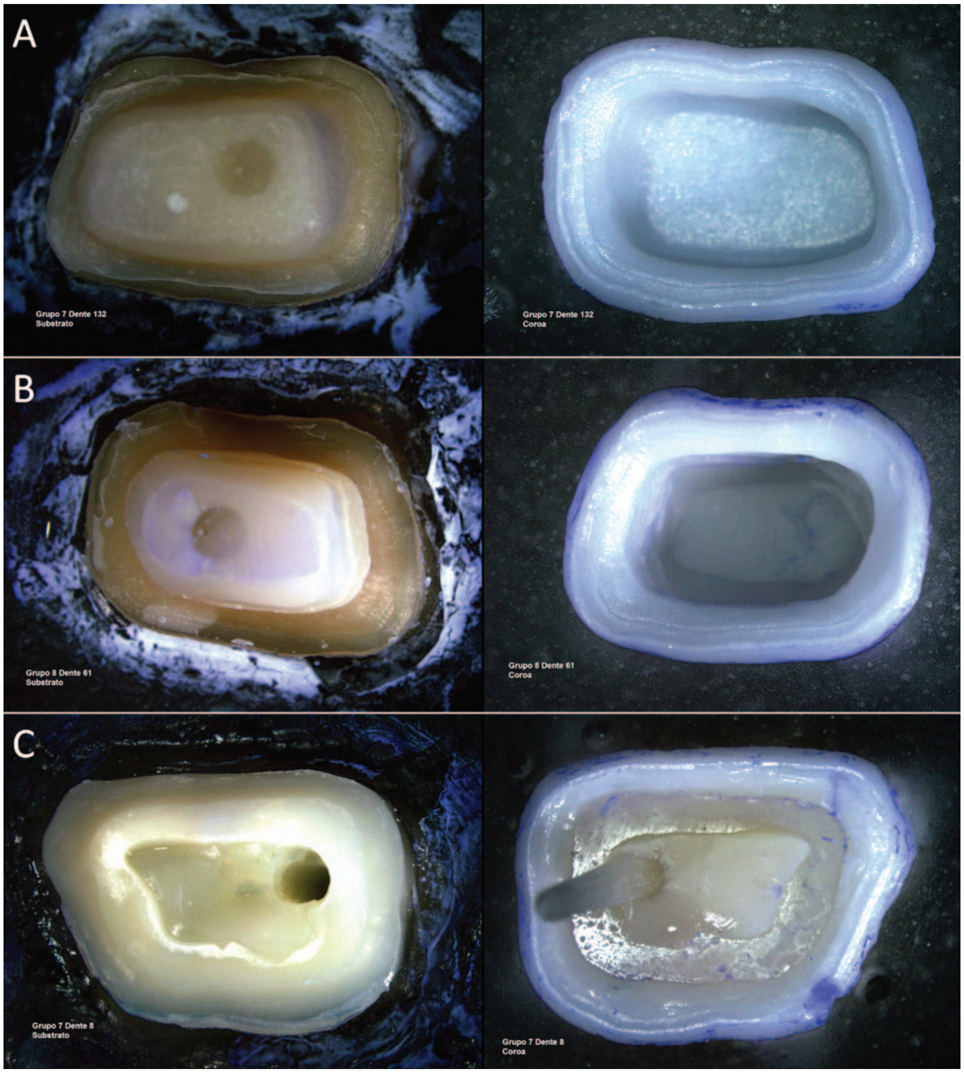

Figure 4. *Photos from stereomicroscopy of the failure types. (A): Over 50% of cement on the substrate, (B): Over 50% of cement on the crown inner surface. (C): Catastrophic failure.*

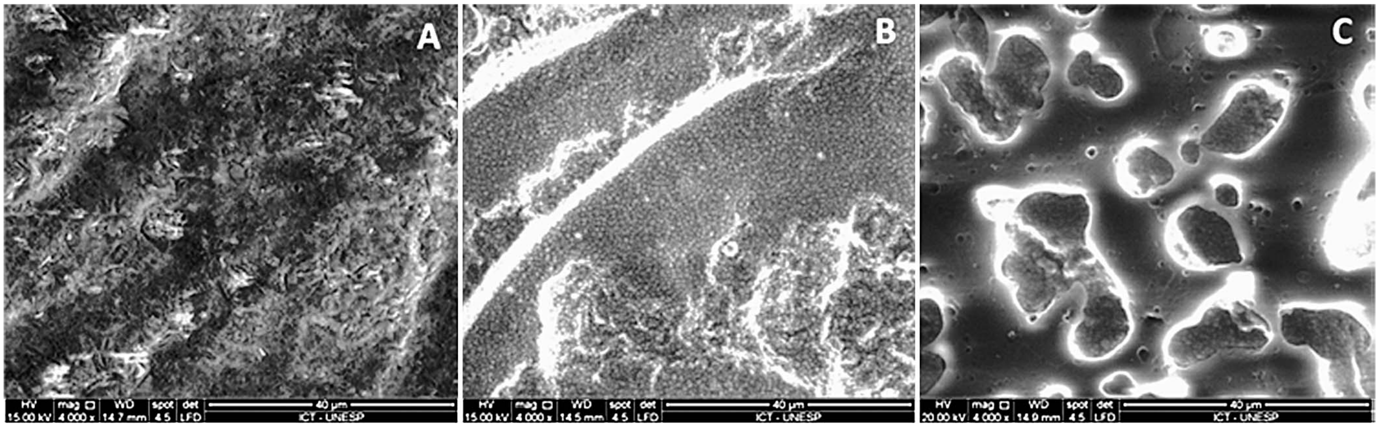

Figure 5. *Representative SEM images of Y-TZP ceramic surfaces. (A): Zirconia without treatment. (B): Zirconia air abraded with 30-μm particles of aluminum oxide coated with silicon. (C): Glazed and etched zirconia surface.*

therefore the second hypothesis was rejected. Silica coating did not increase the retention values of the crowns for the Multilink and RelyX ARC cements when compared to the control group. This fact may be related to the difficulty in applying the silica layer on the zirconia surface since Y-TZP has very high hardness and fracture toughness. Ntala and others[21] found that this surface treatment did not create the micromechanical retention required for efficient adhesive bonding, confirming the findings of the present study. Additionally, according to Borges and others[30] and Oyague and others,[31] airborne particle abrasion using 50 μm of alumina oxide has little effect on the morphological surface features of zirconium dioxide ceramics and does not result in a deep surface modification. In the present study, smaller particles were used (30 μm), which might have prevented surface modification. Perhaps this is the reason why the group cemented with silica coating and Relyx ARC showed the highest amount of remaining cement on the core surface and not on the crown cementation surface. For the Multilink cement, most of the failures were catastrophic, indicating that this group (MultS) probably would show greater retention values if post debonding were avoided.

The vitrification process was efficient only for the Multilink cement, as it resulted in improved retention values when compared to the control groups. However, for the MultV group, most failures were either catastrophic or between the crown and cement with higher amounts of cement remaining on the core surface. As previously mentioned, if the specimens that fractured catastrophically could have been tested, the failures would probably have occurred between the crown and cement because no failures occurred between the core and the cement in the MultV group.

It is probable that the 10-μm-thick[12] glass layer on the inner surface might have caused increased friction between the crown and the preparation walls, leading to higher retention values. In addition, Vanderlei and others[12] showed that one of the limitations of the vitrification technique is the difficulty in standardizing the glaze application inside the Y-TZP infrastructure since the glass layer applied on the intaglio surface of the Y-TZP infrastructure creates a layer thick enough to interfere with seating the infrastructure. Thus, technical improvements need to be tested for reducing the effect on the marginal adaptation.

When all cements without zirconia surface treatment were compared (Table 3), the groups that had the highest retention values were the BIS-GMA- and HEMA-based cements. Thus, the third hypothesis of this study was accepted. Regarding RelyX U100, although its chemical composition containing methacrylated phosphoric esters has not been fully disclosed by the manufacturer, it has been shown that these monomers can bond to ceramic surfaces by means of the same mechanisms previously described for the monomer 10-MDP (also a methacrylated phosphoric acid ester).[32] Yap and others[33] reported that the bonding mechanism of RelyX Unicem is reminiscent of the self-adhesiveness of glass ionomer cements and that a possible improvement in bond strength may occur after cement maturation, over time. However, according to the failure analysis, which shows that the largest amount of cement remained attached in the core, it can be concluded that the bond failure occurred between the cement and the zirconia.

Manufacturers of the glass ionomer based–cement reported that, although this material has a chemical affinity for dentin hydroxyapatite, it has little affinity to the resin composites, which explains their low performance in terms of retention values and failure mode (between cement and composite resin core for all specimens).

In the present study, the manufacturers of the zinc phosphate cement (control group) still claim that this material can be used for cementation of zirconia crowns. It is interesting to note that this cement showed statistically similar retention values when compared to those obtained by the ionomer and self-adhesive resin cements. This retention behavior of the zinc-phosphate cements may be attributed only to the high friction coefficient between the crown and composite resin core walls, as this material does not bond to either the ceramic or the preparation substrate.[34]

One of the limitations of this study was the fact that it was not possible to measure the adhesive area of the preparation in order to calculate the nominal tensile retention stress for all of the ceramic crowns. However, it is believed that the retention force values (in kgf) used by this current investigation were reliable enough once samples were randomized and preparations were standardized. Palacios and others[28] compared the tensile strength (in MPa) and the load for crown pull-out (tensile retention in kg) and showed similar outcomes for both measurement units due to the homogeneity of preparation total areas. Those authors also calculated the average total preparation area for each experimental group and showed that the adhesive areas were similar for the different teeth used.

The clinical relevance of this study is that it simulated different cementation protocols for Y-TZP crowns in an *in vitro* design, as a clinical crown cemented on a tooth reconstructed with resin composite core and a fiber post. Further studies should be conducted to investigate other factors involved in the retention of Y-TZP crowns, such as longitudinal fatigue testing, the evaluation of different cementation strategies, and other surface treatments for the inner surface of Y-TZP frameworks.

CONCLUSION

1) The type of resin cement (BIS-GMA- or HEMA-based) did not affect the crown retention values.
2) The conditioning of the zirconia intaglio surface by the application of a thin low-fusing glass porcelain layer plus silanization was capable of improving the retention force for the HEMA-based cement.
3) For the untreated intaglio surfaces of Y-TZP crowns, resin cements showed significantly higher retention values when compared to those obtained for the self-adhesive resin cement, glass ionomer, and zinc phosphate cements.

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Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Analysis of Anticaries Potential of Pit and Fissures Sealants Containing Amorphous Calcium Phosphate Using Synchrotron Microtomography

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Clinical Relevance

The combination of fluoride and resin sealants containing amorphous calcium phosphate was highly effective at preventing the demineralization of enamel.

SUMMARY

The aim of this study was to analyze the anticaries potential of pit and fissure sealants containing amorphous calcium phosphate (ACP) by synchrotron microtomography. Bovine enamel blocks (4×4 mm; n=50) were selected through surface hardness (Knoop) analysis. Slabs were obtained through cross-

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sections taken 1 mm from the border of the enamel. Five indentations, spaced 100 μm apart, were made 300 μm from the border. Ten specimens were prepared for each tested material (Ultraseal XT plus TM, Aegis, Embrace, Vitremer and Experimental Sealant). The materials were randomly attached to the sectioned surfaces of the enamel blocks and fixed with sticky wax. The specimens were submitted to pH cycling. After that, the surface hardness (SH_1) was determined, and the blocks were submitted to synchrotron microcomputed tomography analysis to calculate the mineral concentration (Δg_{HAp} cm^{-3}) at different areas of the enamel. The comparison between the SH_1 and Δg_{HAp} cm^{-3} showed a correlation for all groups (r=0.840; p<0.001). The fluoride groups presented positive values of Δg_{HAp} cm^{-3}, indicating a mineral gain that was observed mainly in the outer part of the enamel. The ACP showed mineral loss in the outer enamel compared with fluoride groups, although it inhibited the demineralization in the deeper areas of enamel. The combination of two remineralizing agents (fluoride and ACP) was highly effective in preventing demineralization.

INTRODUCTION

Amorphous calcium phosphate (ACP) has been identified as a possible precursor in the formation of hydroxyapatite. Dental applications based on the unique characteristics of ACP have been proposed, and it has been shown that the properties of ACP are enhanced when used with similar products that have anti-demineralizing and remineralizing potential.[1] The systems developed use casein phosphopeptides (CPPs) to stabilize the calcium phosphate ions at high concentrations; these include amorphous nanocomplexes designated CPP-ACP on the enamel surface.[2] Incorporating CPP-ACP into glass-ionomer cements improved the anticariogenic potential of this material without adversely affecting its mechanical properties.[3,4] Recent studies show that composites containing ACP can release supersaturated levels of calcium and phosphate ions in proportions favorable for apatite formation.[5-8] In these composites, the addition of ACP led to failures due to degraded mechanical strength; thus, they are not indicated as restorative or lining materials but can be adequate as pit and fissure sealants.[5,9]

As a sealant, two studies (*in vitro* and *in situ*) showed that the ACP sealant presented the same capacity of remineralization as the fluoride sealant.[7,10] When the sealant product contained ACP and fluoride (ACP-F), there was no improvement in its remineralizing effect.[7] However, it is important to determine if the ACP sealant has the ability to inhibit enamel demineralization because caries is a dynamic process that involves demineralization and remineralization. If it is determined that sealants with ACP-F act as a favorable alternative process for remineralization of the enamel compared with resin agents that contain only fluoride, practitioners would have an additional method for preventing dental caries. The purpose of this study was to analyze the anticaries potential of an ACP sealant and an ACP-F sealant using a pH-cycling model and synchrotron microtomography.

METHODS AND MATERIALS

Preparation and Selection of Enamel Blocks

Enamel blocks (4 mm×4 mm×3 mm; n=50) were obtained from bovine incisor teeth that were stored in 2% formaldehyde solution with a pH of 7.0 for 30 days.[11] The enamel surface of the blocks was then serially polished, and the slabs were cross-sectioned at 1 mm from the border (Figure 1A), resulting in specimens with an area of 12 mm^2.[11] The blocks were subjected to surface hardness (Knoop) analysis (SH) using a microhardness tester (Shimadzu MicroHardness Tester HMV-2000, Shimadzu Corp, Kyoto, Japan) with a Knoop diamond under a 25g load for 10 seconds.[11] Five indentations spaced 100 μm from each other were made at a distance of 300 μm from the enamel sectioned border (Figure 1B). Enamel blocks with hardness values between 330 and 370 kgf/mm^2 were selected.

Sample Preparation and Enamel Block Adaptation

Ten samples were prepared for each tested material (Table 1) using a metallic matrix (4 mm×2 mm×1 mm) (Figure 1C) following the manufacturer's instructions, with the exception of the Vitremer (3M ESPE, St Paul, MN, USA), which had a diluted mix at 1/4 the powder to liquid ratio.[11,12] Polymerization of the materials was performed with a VIP unit (BISCO, Schaumburg, IL, USA) for 40 seconds on both sides of the specimen, using a light intensity of 500 mW/cm^2. After the sample preparation (Figure 1D), the materials were randomly attached to the sectioned surfaces of the bovine enamel blocks (Figure 1E) and fixed with sticky wax (Kota Industria and Comércio Ltda, São Paulo, Brazil) (Figure 1F).[11] The specimens (enamel block +

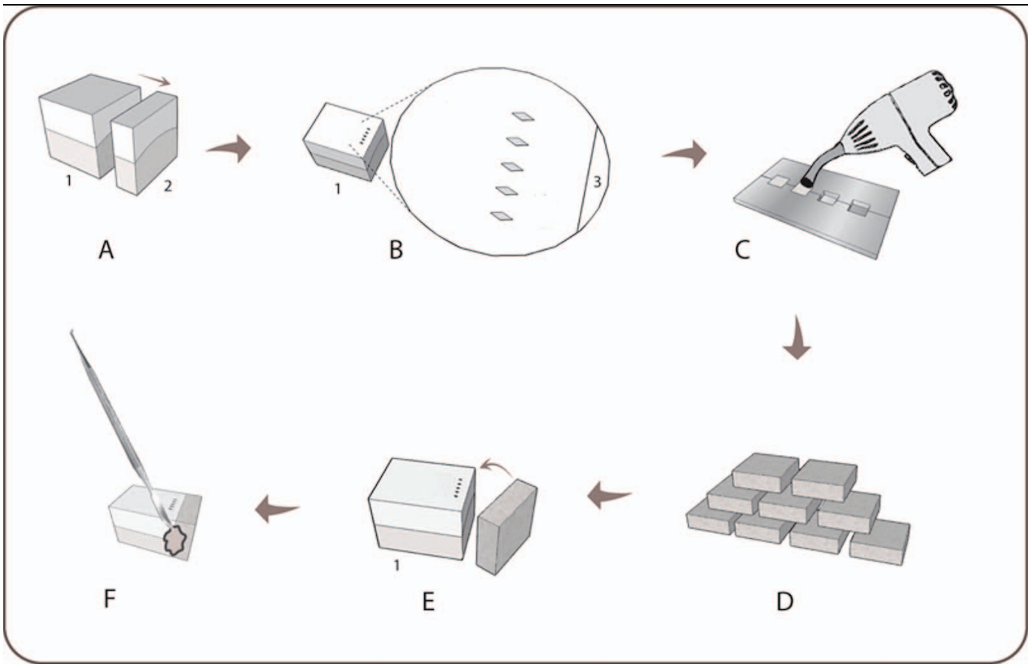

Figure 1. *Schematic presentation. (A) Section of the block (1. block 3×4 mm used in the research; 2. Piece of the block 1×4 mm discarded). (B) Five indentations at 300 μm from the enamel sectioned border (3). (C) Polymerization of sample. (D) Samples. (E) Samples adapted onto the enamel blocks (3). (F) Samples fixed with wax.*[11]

sample) were then coated with an acid-resistant varnish, except for the enamel and sample surface.

pH Cycling

The effect of the material in interfering with the dynamic caries process was evaluated. To simulate the demineralization and remineralization process *in vitro*, all specimens were immersed in demineralizing solution for six hours (2.0 mmol/L Ca and P, 0.075 mol/L acetate buffer, 0.04 ppm F, 2.2 mL/mm^2 of the enamel surface, pH 4.7) and remineralizing solution for 18 hours (1.5 mmol/L Ca, 0.9 mmol/L P, 0.15 mol/L KCl, 0.02 mol/L Tris buffer, 0.05 ppm F, 1.1 mL/mm^2 of the enamel surface, pH 7.0) for five days at 37°C. The specimens were then submerged in the remineralizing solution for an additional two days before the surface hardness analysis.[11]

Surface Hardness Analysis

The surface hardness analysis was performed using a Shimadzu HMV-2000 (Shimadzu Corp) microhardness tester and a Knoop diamond under a 25g load for 10 seconds. Five indentations spaced 100 μm from each other were made on the slab surface at 300 μm from the sectioned enamel border (Figure 1B).[11] After the pH cycling, the final surface hardness (SH_1) was measured, following the aforementioned methodology.

Analysis of Synchrotron Microcomputed Tomography

Five blocks of each group were sectioned longitudinally and transversely; the samples (1.5 mm×2.0 mm) were them submitted to synchrotron microcomputed tomography at an Advanced Photon

Table 1: *Identification of the Tested Material*

Material	Manufaturer	Classification	Batch No.	Group
Ultraseal XT plus	Ultradent Products Inc, South Jordan, UT, USA	Light-cured resin sealant	B 0997	Resin no-F
Aegis	Bosworth Company, Skokie, IL, USA	Light-cured ACP resin sealant	0407-397	Resin ACP
Experimental sealant	Bosworth Company, Skokie, IL, USA	Light-cured ACP and F resin sealant	HJB6-203A	Resin ACP-F
Embrace	Pulpdent Corporation, Watertown, MA, USA	Light-cured F resin sealant	040923	Resin F
Vitremer	3M/ESPE, St Paul, MN, USA	Resin-modified glass ionomer cement	22015	Ionomer

Abbreviations: ACP, amorphous calcium phosphate; F, fluoride.

Table 2: *Analysis of Surface Hardness (SH_1), Integrated Loss of Subsurface Mineral (Δg_{HAp} cm^{-3}), and Integrated Differential Mineral Concentration (ΔIML) According to Groups*[a]

Groups	**SH_1 (kgf/mm^2)**	**Δg_{HAp} cm^{-3}**	**ΔIML, g_{HAp} cm^{-3}**	
			Zone A (2.8–33.6 μm)	**Zone B (36.4–89.6 μm)**
Resin no-F	47.9^{a} (20.8)	-15.6^{a} (4.5)	-19.8^{Aa} (5.5)	-7.9^{Aa} (10.2)
Resin F	285.6^{b} (21.3)	0.3^{b} (1.9)	0.7^{Ab} (4.0)	$0.6^{Aa,b}$ (6.7)
Resin ACP	94.7^{c} (45.1)	-7.3^{c} (1.3)	-3.0^{Ab} (4.4)	3.8^{Ab} (9.4)
Resin ACP-F	213.9^{d} (37.0)	2.5^{b} (3.7)	1.8^{Ab} (4.6)	$-1.0^{Aa,b}$ (3.9)
Ionomer	288.9^{b} (19.8)	10.5^{d} (2.3)	5.4^{Ab} (3.8)	2.6^{Ab} (5.4)

Abbreviations: ACP, amorphous calcium phosphate; F, fluoride.

[a] Distinct superscript lowercase letters indicate statistical significance in each analysis (Student-Newman-Keuls test; $p<0.05$). Distinct superscript capital letters indicate the differences between zones A and B in each line (Student-Newman-Keuls test; $p<0.05$).

Source 2-BM bending magnet station (Argonne National Laboratory, Argonne, IL, USA). X-ray photons with an energy of 20 keV were provided by a double multilayer monochromator.[13] The detector system consisted of a 12-bit, CoolSNAP 2K × 2K CCD camera (Princeton Instrument, New Jersey, USA) coupled with an optical lens (2.5×) to a CdWO4 single-crystal phosphor. Views were recorded every 0.25° from 0° to 180° and were normalized for the detector and beam nonuniformities. Specimens were reconstructed on a 2K × 2K grid of isotropic voxels, side length ~2.8 μm. The analysis was based on mineral concentrations calculated from the linear attenuation coefficient (μ) and described as mass of pure hydroxyapatite (ρ=3.15 g cm^{-3}) per unit volume of tissue (g_{HAp} cm^{-3}).[14]

The integrated area under the curve (cross-sectional mineral profiles into the enamel; Figure 1a), using mineral concentration values (g_{HAp} cm^{-3}) was calculated and the integrated loss of subsurface mineral (Δ_{gHAp} cm^{-3}) determined.[15] The differential mineral concentration profiles (Figure 1b) for the materials and mineral concentrations of sound enamel (ie, g_{HAp} cm^{-3} value of materials minus 2.40 g_{HAp} cm^{-3}) at each of the material groups were also calculated. These differential profiles were then integrated over two depth zones in the lesion and underlying sound enamel to yield the ΔIML values.[16] Zone boundaries were selected at 33.6 and 89.6 μm to highlight differences in the mineral concentration at different depths.

Statistical Analysis

Analyses were performed using the SigmaPlot software (version 12.0, Systat Software Incorporation, San Jose, CA, USA), and the level of statistical significance was established at 5%. After confirmation of the normal (Shapiro-Wilk) and homogeneous (Cochran) distributions, the data from the SH_1 and Δg_{HAp} cm^{-3} were submitted to one-way analysis of variance, followed by the Student-Newman-Keuls test. The results from the ΔIML were submitted to two-way analysis of variance, followed by the Student-Newman-Keuls test. The Pearson's correlation coefficient was calculated for the SH_1 and Δg_{HAp} cm^{-3}.

RESULTS

The surface hardness data of the enamel before the pH cycling exhibited similar mean values (352.1–356.0 kgf/mm^2; p=0.755).The resin no-F group showed the lowest values of hardness after the pH-cycling (p=0.001) when compared to the other groups (Table 2). The resin F and ionomer groups showed similar values of hardness (p=0.807) and were higher than the other groups ($p<0.001$).

The mean of mineral concentration values for sound bovine enamel of the blocks was 2.40 ± 0.06 (2.28–2.54) g_{HAp} cm^{-3}. The results of the Δg_{HAp} cm^{-3} (Table 2) showed that treatment with ionomer, resin ACP-F, and resin F did not present with mineral loss. Resin ACP reduced the mineral loss compared with the resin no-F ($p<0.001$). The combined fluoride/ACP (resin ACP-F group) had an improved resistance against mineral loss, presenting similar values when compared with the resin F group ($p>0.264$).

The profiles of mineral concentration (Figure 2a) showed a subsurface lesion from the resin no-F and resin ACP. The enamel closer (2.8–5.6 μm) to ionomer (2.82 ± 0.25 g_{HAp} cm^{-3}), resin ACP-F (2.75 ± 0.20 g_{HAp} cm^{-3}), resin F (2.61 ± 0.08 g_{HAp} cm^{-3}) and resin ACP (2.61 ± 0.20 g_{HAp} cm^{-3}) presented hypermineralization at 2.8 μm (p=0.133). These groups did not show the formation of a subsurface lesion, except for the resin ACP group. The resins with fluoride and/or ACP presented the same results (p=0.156) at the outer enamel (zone A). However, in

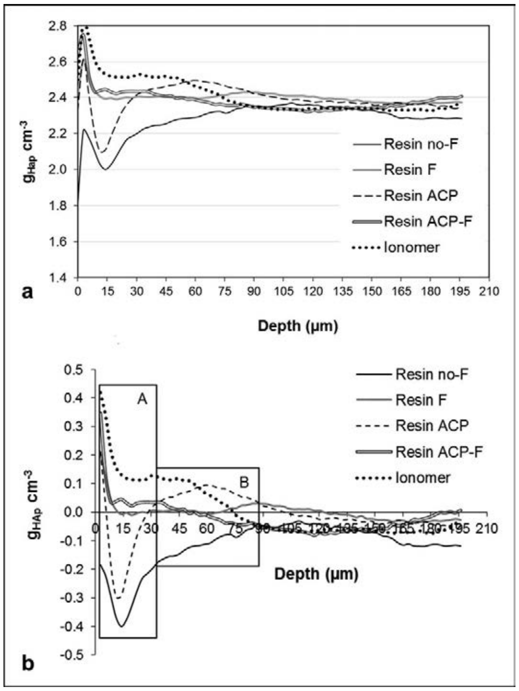

Figure 2. *(a) Depth profiles of mineral concentration (g_{HAp} cm^{-3}) in lesions for each group. (b) Differential mineral concentration profiles (g_{HAp} cm^{-3} vs depth) calculated by subtracting resin profiles from sound enamel value (2.40 g_{HAp} cm^{-3}). Positive values thus indicate a higher mineral concentration at the given depth in the resin groups than the sound enamel, and vice versa. Differential profiles were integrated into each of the marked zones (A–B) to give depth-dependent ΔIML values.*

the depth of the lesion (zone B, 36.4–89.6 µm), the resin ACP showed higher mineral concentration (p=0.038) along with the ionomer (p=0.049) when compared with the resin no-F (Table 2 and Figure 2b).

DISCUSSION

Because ACP is capable of increasing the calcium and phosphate concentrations within a lesion, it has been added to dental products as a remineralizing agent. The addition of ACP in sealant improves the enamel remineralization, as does a fluoride sealant, even presenting different forms of apatite deposition.[7,10] The capacity of ACP deposits on the enamel surface to inhibit the enamel demineralization has not yet been studied. Besides the *in vitro* demineralization produced in the present study, marginal adaptation failed between the material and enamel, a problem that can occur *in vivo*. The present study showed that ACP released by the sealant led to limited effects against demineralization, but combining it with fluoride can improve its effects. For this study, CPPs were not used to stabilize fluoride and ACP.

The calcium and phosphate release from the resin ACP led to supersaturation of the biofilm with respect to the hydroxyapatite as observed in previous research.[8] In the present study, these products were able to reduce the mineral loss, thus increasing the mineral concentration on the enamel surface to the same degree as the fluoride materials. This effect is mainly a product of the ionic activity product of the $CaHPO_4^0$ and the buffering capacity of the ACP.[8,17] These mineral depositions do not contribute to recovery of the prismatic structure and consequent mechanical properties. The mineral deposition produced by the ACP sealant between 2.8 and 5.6 µm was able to attenuate the X-ray photons but it seems this did not lead to the mechanical restructuring of the prismatic structure as the hardness was lower for this group. Moreover, the supersaturation of the biofilm with respect to the hydroxyapatite produced by resin ACP was not able to reduce the mineral loss in the enamel subsurface at a depth between 8.4 µm and 28.0 µm, where the acid activity should be more intense. However, the ACP can produce a great flux of calcium and phosphate in the deeper part of the enamel,[18] leading to a great mineral gain as observed in the present study in the depth of 36.4 µm to 89.6 µm.

Nevertheless, the fluoride materials are able to increase the biofilm saturation with respect to hydroxiapatite and fluorapatite.[8] This can explain the higher mineral concentration of the enamel, mainly at the enamel surface. Greater ionic activity does not occur just for the $CaHPO_4^0$ product but also for the HF^0.[8,17] This leads to the effect being mainly on the surface.[18] The mineral deposited in the enamel surface is harder in the presence of fluoride, but there was not a total recovery of the surface hardness. As the ionomer group was prepared with a powder/liquid ratio (¼:1) that leads to higher fluoride release,[11,12] this can explain the greater anticaries effects.[11]

Adding fluoride to resin with ACP (ACP-F resin group) improved the capacity to inhibit the demineralization to a similar degree as the fluoride materials. Probably, this is because the resin ACP-F led to an increase in saturation with respect to the hydroxyapatite and fluorapatite. Because the ACP in the resin materials is stabilized by hybridization with ions such as Zr^{2+} or SiO^{4-},[5,6] adding sodium fluoride is possible without ACP to react to the fluoride in the composite body. These associations can present a greater ionic activity of the $CaHPO_4^0$ and HF^0 products which is important mainly in the remineralization of the enamel.[8,17] This might have produced a greater effect on the process of remineralization than demineralization, even with increased cariogenic challenge. The data from the mineral concentration profile (Figure 2) support this observation as there was an increase in the Δg_{HAp} cm^{-3} values. This can explain the hypermineralization at 30 μm from the enamel surface.

CONCLUSION

The present study showed that the ACP inhibits demineralization in the deeper part of enamel, whereas the fluoride products had a greater effect at the outer part of the enamel. The combination of two remineralizing agents (fluoride and ACP) was highly effective in preventing demineralization.

Conflict of Interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Efficacy of Do-It-Yourself Whitening as Compared to Conventional Tooth Whitening Modalities: An In Vitro Study

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Y Li

Clinical Relevance

Professionally applied in-office whitening, professionally dispensed patient-applied home whitening, and the use of an over-the-counter product are effective in changing tooth color toward a lighter shade, whereas do-it-yourself whitening is not an effective tooth whitening modality.

SUMMARY

Aim: To evaluate the efficacy of do-it-yourself (DIY) whitening as compared to conventional tooth whitening modalities using different shade assessment tools.

Methods and Materials: Extracted human molars (120) were randomly distributed to six groups (n=20). Whitening was performed according to manufacturer's directions for over-the-counter, dentist-dispensed for home use, and in-office whitening. DIY whitening consisted of a strawberry and baking soda mix. Additionally, negative and positive controls were used. Two evaluators used the Vita Classical (VC) and Vita Bleachedguide 3D-Master with interpolated numbers (BGi) for visual assessment at baseline and one-week, one-month, and three-month postwhitening. Instrumental measurements were performed with a spectrophotometer. Kruskal-Wallis procedure was used to assess color changes among groups and intraclass correlation (ICC) to evaluate agreement between evaluators.

Results: DIY exhibited lower color change (ΔSGU_{VC}, ΔSGU_{BGi}, ΔE^*, where SGU = shade guide unit and E = overall color change) compared to other whitening groups at all time points ($p<0.05$). ICC demonstrated very

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good agreement between evaluators with VC and BGi at each time point. Both shade guides were related with each other and strongly related to instrumental measurements ($p<0.05$).

Conclusions: DIY whitening was the least effective whitening modality. Both VC and BGi are related with each other and have good correlation with instrumental measurements.

INTRODUCTION

Demand for tooth whitening has been building and growing for more than a decade as people envision and desire a "Hollywood smile." Tooth whitening now represents the most common elective dental procedure[1] and has proven to be safe and effective when supervised by a dentist.[2] More than 1 million Americans whiten their teeth annually, driving nearly $600 million in revenues for dental offices.[1] The high demand is also reflected by the wide range of whitening modalities available: professionally applied in-office whitening; professionally dispensed patient-applied home whitening, and over-the-counter (OTC) products.[2]

The penchant to do it yourself has swept through myriad websites promoting the use of at-home whitening remedies,[3] including rubbing crushed strawberries or apples on the teeth. Thus, a new category has emerged that can be described as do-it-yourself (DIY) whitening using natural ingredients.[4] The advocated use of fruits such as strawberries and apples is based solely on anecdotal evidence with the rationale that they contain malic acid. Considering the current evidence of the erosion potential of fruit-containing beverages and smoothies[5-8] and controversies on the safety of DIY methods,[9] appropriate cautions for their use in tooth whitening is paramount.

Tooth whitening efficacy has been evaluated visually with clinically accepted shade guides and instrumentally with electronic color measuring devices. The Vita Classical (VC; Vita Zahnfabrik, Bad Säckingen, Germany) developed in 1956 is one of the most commonly used shade guides. However, its use in monitoring tooth whitening has been disputed due to a lack of logical order, uniform color distribution, and light shade tabs, thus limiting the design of whitening studies.[10] The Vita Bleached-guide 3D-Master (BG; Vita Zahnfabrik) was introduced in 2007 to increase the reliability and validity of visual color assessments by including more whitening shade tabs, uniform distribution between the tabs, and a visually perceivable light to dark value order.[11] The BG tabs are now available with new markings, with each tab marked with odd numbers from 1 to 29 and with "interpolated" even numbers in between. The main advantage of the interpolated BG (BGi) that has been reported is that the distribution of the tabs better corresponds to the American Dental Association (ADA) equation that 1 ccu = 1 SGU = 1 ΔE^* (ccu = color change unit; SGU shade guide unit; ΔE^* = overall color change).[12]

With heightened consumer interest in whiter teeth, it is the responsibility of the dental profession to educate the patient and the public about the efficacy and adverse effects of different tooth whitening modalities, suggest or provide appropriate whitening options based on patient's needs and preference, and establish reliable and valid monitoring tools for the whitening process. According to the current literature, there seems to be a lack in knowledge of comparing whitening efficacy of all four whitening modalities. There is also a lack of information on the correlation of the newly introduced interpolated shade guide with conventional shade guides.

The purpose of this study was to compare the efficacy of DIY whitening as compared to conventional whitening modalities with respect to tooth color change. Tooth color change was monitored with three different shade evaluation methods: visual assessment using VC and Interpolated BGi and spectrophotometric color assessment with the Vita Easyshade Compact (ES; Vita Zahnfabrik).

The null hypotheses to be tested were the following. First, there will be no difference in tooth color change among the four different whitening modalities. Second, there will be no differences in agreement between the two evaluators using VC and BGi. Third, there will be no correlation between visual and instrumental color measurements.

METHODS AND MATERIALS

Sample Selection and Preparation

Extracted sound human third molars without identifiers (120) were collected and stored in 0.2% sodium azide solution at 4°C. Teeth were cleaned of gross debris and placed in artificial saliva for 24 hours at 37°C prior to initiating the experiment. Artificial saliva was prepared and replaced daily according to the modified Fusayama solution as described in ANSI/ADA Specification 41.[13]

Table 1: *Active Ingredient, pH, and Application Regimen by Group*

Group	Concentration	pH	Application Regimen
NC: water of grade 3		7.0	One application (60 min), at 35°C
DIY: strawberry mix	CA	7.2	Three applications (5 min each) at 5-d intervals
OTC: Crest 3D Intensive	9.5% HP	6.0	One daily application (2 h) for 7 d
HW: Opalescence PF	10% CP	7.4	One daily application (6 h) for 14 d
OW: Zoom WhiteSpeed	25% HP	7.2	Three applications (45 min each) at 5-d intervals
PC: citric acid	1.0% CA	3.9	One application (60 min), at 35°C

Abbreviations: CA, citric acid; CP, carbamide peroxide; DIY, do-it-yourself; HP, hydrogen peroxide, HW, home whitening; NC, negative control; OTC, over-the-counter; OW, office whitening; PC, positive control.

Experimental Groups

Specimens were randomly distributed into six groups of 20 specimens each and mounted on acrylic molds to expose the crown portion for the different whitening treatments. The active ingredient, pH, and application regimen by group are summarized in Table 1. The treatment regimen for each group followed manufacturers' instructions or recommendations for typical use. DIY represented a method using a puree of strawberry (15 g) mixed with baking soda (2.5 g; Arm & Hammer Baking Soda, Church & Dwight Co, Inc, Princeton, NJ, USA) for five minutes followed by brushing with a soft toothbrush (Colgate Oral Pharmaceuticals, Inc, New York, NY, USA) for 30 seconds. The procedure was repeated two more times at five-day intervals. Group OTC received daily applications of whitening strips for two hours (Crest 3D Intensive, Crest Pro Health, Procter & Gamble, Cincinnati, OH, USA) for seven days. Group HW was treated with a 10% carbamide peroxide gel (Opalescence PF, Ultradent Products Inc, South Jordan, UT, USA) placed in a custom fabricated tray for six hours for 14 days (0.12 ml/tooth). Group OW represented the professionally applied group treated with 25% hydrogen peroxide (Philips Zoom White Speed, Philips Oral Healthcare, Los Angeles, CA, USA) with three applications for 45 minutes using light activation. The whitening material was replenished every 15 minutes according to the manufacturer's instructions. The negative (NC) and positive control (PC) groups were treated according to the International Organization for Standardization (ISO) 28399 protocol with grade 3 water and 1.0% citric acid for 60 minutes at 35°C, respectively.[14]

Tooth Color Change Assessment

Two shade guides, the VC and BGi, were used for visual color assessment (Figures 1 and 2). Measurements were performed under a color-controlled light box (MM 4e GTI Mini Matcher, GTI Graphic Technology, Inc, Newburgh, NY, USA) at CIE D_{65}, a color temperature of 6500 K, and light intensity of ≈1200 lux by two evaluators with superior color matching competency (ISO/TR 28642).[15] Results were expressed in difference of shade guide units (ΔSGU) for the respective shade guides. Instrumental color measurements were performed on the middle third of the buccal surface using a contact-type intraoral spectrophotometer (ES), Vita Easyshade Compact, with the use of a custom-fabricated clear jig for repeatable measurements (Figure 3). The overall color change as measured with the

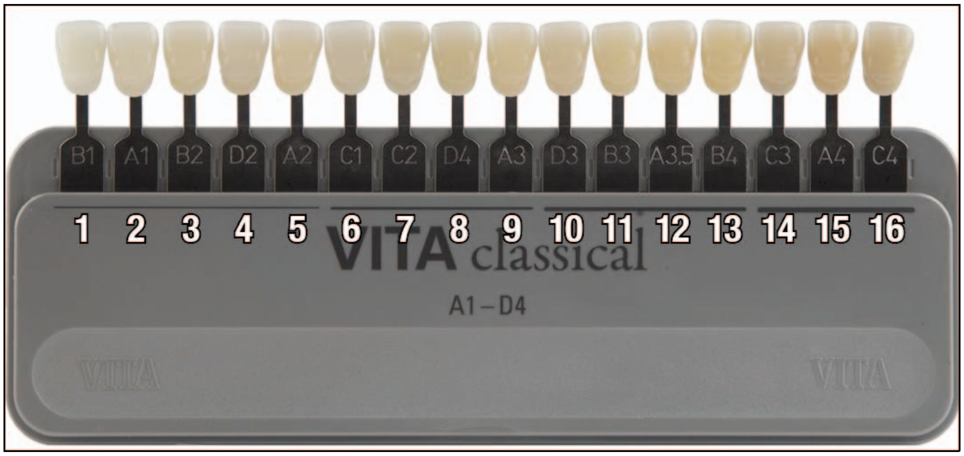

Figure 1. *Value-oriented Vita Classical Shade Guide.*

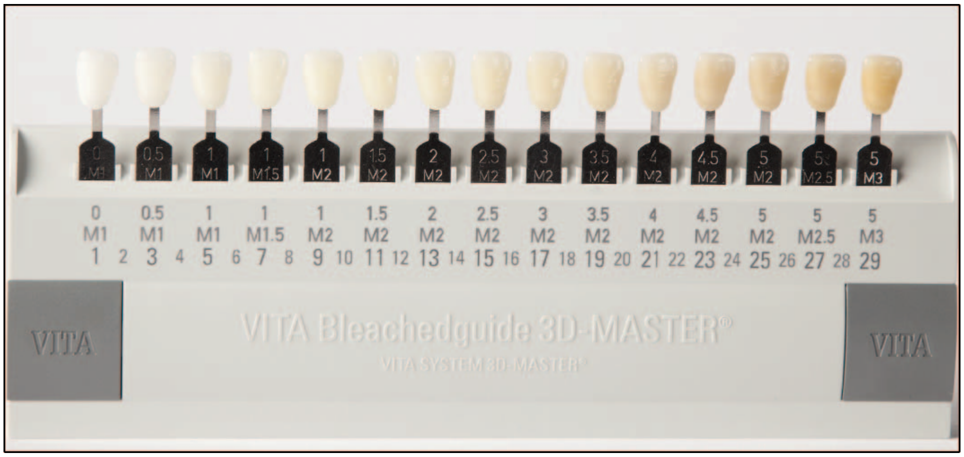

Figure 2. *Vita Bleachedguide 3D-Master with interpolated numbers.*

spectrophotometer was expressed as ΔE* from the Commission Internationale de l'Eclairage. The following equation was used:

$$\Delta E* = [(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2]^{1/2}$$

The color differences were calculated relative to baseline color parameters (L^*_1, a^*_1, b^*_1). Measurements were performed with the VC, BGi, and ES at baseline (T_1), one-week postwhitening (T_2) and one-month (T_3), and three-month postwhitening (T_4).

Statistical Analysis

Measurements of color change included ΔSGU_{VC}, ΔSGU_{BGi}, and ΔE*. The Kruskal-Wallis procedure was used to determine significant differences in color change among the groups. Friedman's test was used to evaluate differences in color change across the different time points. Intraclass correlation (ICC) was used to assess the agreement between the two evaluators using the VC and BGi. Correlations between visual and instrumental measurements were assessed with Pearson correlations. Tests of hypotheses were two sided with an alpha level of 0.05. Analysis was conducted with SAS version 9.2 (SAS Institute, Cary, NC, USA).

RESULTS

Visual and instrumental color changes by groups at three time points are summarized in Table 2. Baseline color parameters of the six groups were not different with the visual and the instrumental methods ($p>0.05$). However, color changes among the groups were significantly different at all time periods for the visual and instrumental methods ($p<0.05$). Post hoc tests (Scheffe) for VC, BGi, and ES at one week (T2-T1), one month (T3-T1), and three months (T4-T1) showed that there were significantly greater color changes in groups OTC, HW, and OW than in groups NC, DIY, and PC. ($p<0.05$). At three months, the greatest overall color change (ΔE*) was observed for group OW ($p<0.05$; Figure 4). However, visual assessment showed no difference among OTC, HW, and OW at three-month

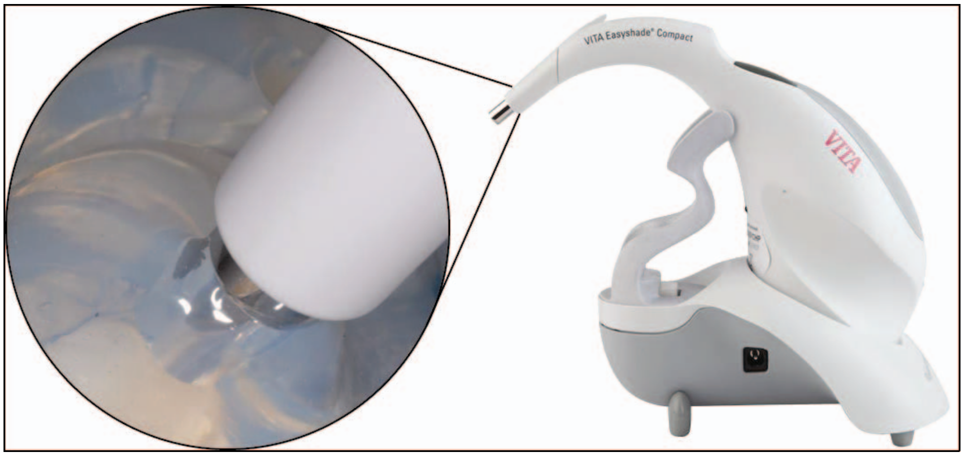

Figure 3. *Vita Easyshade Compact and the use of custom-fabricated clear jigs for repeated measurements.*

Table 2: *Visual and Instrumental Color Changes (Mean [SD]) by Groups at Three Time Points*[a]

Group	1 wk (T2-T1)			1 mt (T3-T1)			3 mt (T4-T1)		
	ΔSGU_{VC}	ΔSGU_{BGi}	ΔE^*	ΔSGU_{VC}	ΔSGU_{BGi}	ΔE^*	ΔSGU_{VC}	ΔSGU_{BGi}	ΔE^*
NC	0.4(0.8) A	−0.6 (1.5) AC	3.2 (2.0) A	1.3 (1.4) A	0.7 (1.8) A	2.2 (1.5) AC	1.4 (1.3) A	0.1 (1.5) A	2.3 (1.0) A
DIY	0.5 (1.1) A	1.0 (1.2) A	4.0 (1.9) A	−0.1 (1.8) AC	−1.1 (1.4) A	4.9 (1.9) AD	−0.1 (1.3) A	1.0 (2.3) A	2.4 (1.4) A
OTC	7.4 (2.4) B	11.0 (2.2) B	10.0 (2.6) C	8.1 (2.0) B	11.5 (2.5) B	14.6 (2.8) B	8.6 (2.3) B	12.0 (2.1) B	14.0 (3.0) B
HW	7.7 (3.0) B	11.6 (2.3) B	13.8 (2.4) D	7.7 (3.0) B	11.4 (2.4) B	15.7 (2.8) B	7.5 (3.0) B	12.0 (2.0) B	15.3 (2.4) B
OW	7.7 (1.9) B	12.7 (2.0) B	17.3 (3.3) E	8.0 (2.0) B	13.5 (1.9) B	18.1 (3.4) B	8.0 (2.0) B	13.6 (2.1) B	17.6 (2.8) C
PC	1.1 (1.2) A	1.6 (2.5) AD	5.1 (2.4) A	2.0 (1.4) AD	1.7 (1.9) A	4.6 (2.1) A	1.4 (1.8) A	1.4 (2.0) A	2.53 (1.0) A

Abbreviations: DIY, do-it-yourself; HW, home whitening; NC, negative control; OTC, over-the-counter; OW; office whitening; PC, positive control.
[a] *Within columns, different letters indicate means that are statistically different* ($p<0.05$).

evaluation ($p>0.05$). At three months, NC, DIY, and PC showed an overall color change of 2.3, 2.4, and 2.53, respectively, which is less than the perceptibility threshold of ΔE 2.6 units.[16]

The levels of ICC demonstrated high agreement between the two evaluators using both the VC and the BGi. It is noteworthy that the agreement at baseline (T1) was significantly lower than the agreement observed at T2-T4 for both BGi and VC (Table 3). Assuming linear correlation, the shade guides were strongly and positively related with each other. Both shade guides were strongly related with the instrumental measurements (Table 4).

DISCUSSION

This is the first study that compared the efficacy of a DIY whitening regimen as compared to three conventional whitening modalities. The result of this study is partially limited by the use of extracted human teeth, which will inherently differ from vital teeth in the oral environment. Based on the results, the first null hypothesis was rejected, as significant differences in color change were detected among the four whitening modalities. The second hypothesis was retained; there was no significant difference in agreement between the two evaluators when comparing both shade guides. The data support the rejection of the third hypothesis because of strong correlations between the shade guides and the instrumental measurements.

Several studies have compared the efficacy of OTC products, home whitening, and in-office whitening.[17-19] According to a clinical study that compared three different whitening techniques with respect to the whitening times required to achieve a defined level of whitening, the cycles required increased from office whitening to home whitening to OTC products. That is, one in-office session was comparable to approximately seven days of home whitening and 16 days of using an OTC product.[18] Another clinical study found that home whitening and in-office whitening were superior to OTC whitening

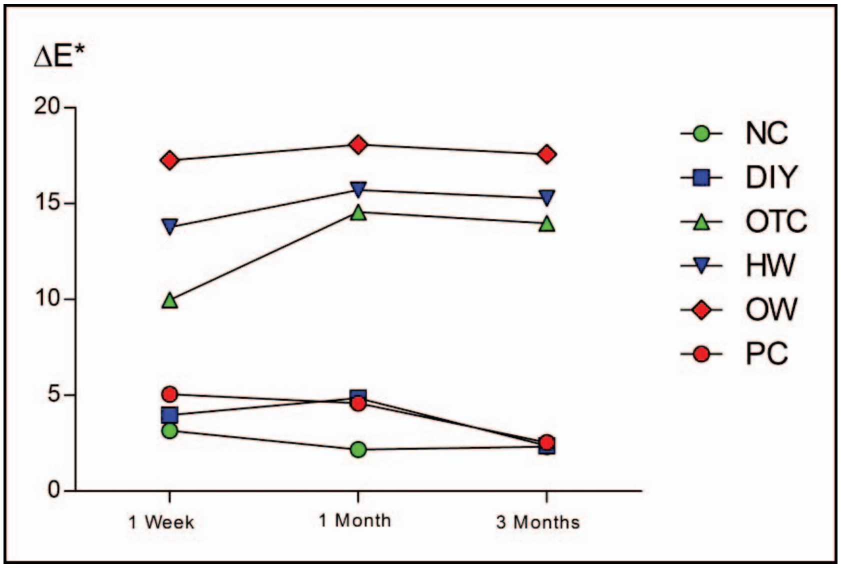

Figure 4. *Overall color change (ΔE*) by group at different time points.*

Table 3: *Agreement Between the Two Evaluators Using VC and BGi*

Shade Guide	Time	ICC	Confidence Interval	
			Lower Bound	Upper Bound
VC	Baseline (T1)	0.777	0.674	0.847
	1 wk (T2)	0.924	0.889	0.948
	1 mo (T3)	0.95	0.868	0.975
	3 mo (T4)	0.954	0.93	0.969
BGi	Baseline (T1)	0.86	0.645	0.93
	1 wk (T2)	0.966	0.946	0.977
	1 mo (T3)	0.984	0.975	0.989
	3 mo (T4)	0.982	0.973	0.988

Abbreviation: ICC, intraclass correlation.

products when followed for up to three months.[19] Our study results showed that the whitening efficacy of Crest Whitestrips, an OTC product, was comparable to professionally dispensed home whitening and professionally applied in-office whitening for up to three months postwhitening when evaluated visually with shade guides. The varying results compared to previous studies may be explained by the difference in study design (e.g., *in vivo* vs *in vitro*) and the difference in whitening products employed. It is important to point out that the change from original Whitestrips using a polymer-based hydrogel system to the new advanced seal technology with an improved seal and increased hydrogen peroxide concentrations may have affected the study results.[20]

DIY whitening with strawberries as well as the positive control group using 1.0% citric acid was not effective in whitening, which showed that color change is not due to the acid component in fruits.

Agreement between the two evaluators was very good for both shade guides VC and the BGi. This is contrary to a study that showed superior performance assessment with the Linearguide 3D-Master compared to the Vita Classical.[21] This can be partly explained by the limitation of the lack of lighter shade tabs in the VC. After specimens were bleached, the only choice was the B1 tab, resulting in high agreement between the two evaluators using the VC. Thus, it is noteworthy to point out that ICC was higher for BGi than VC at all time points, although it was not statistically significant.

Table 4: *Correlations Between Visual and Instrumental Measurements*

	VC	BGi	ΔE*
VC			
Pearson correlation	1	0.896**	0.828**
p-value		0	0
BGi			
Pearson correlation	0.896**	1	0.890**
p-value	0		0
ΔE*			
Pearson correlation	0.828**	0.890**	1
p-value	0	0	

*** Correlation is significant at the 0.01 level, N=360 in all instances.*

The results of this study demonstrated a good correlation between the two shade guides and the instrumental measurements. This supports the use of both shade guides as acceptable tooth shade guides and the use of electronic color measuring instruments for laboratory assessment of tooth whitening efficacy, as described in ADA acceptance guidelines and ISO Standards.[14,22,23] It also suggests that, whenever possible, both visual and instrumental methods should be used, as they complement each other.[24]

Within the limitations of the study, the data provided important information about the efficacy of different whitening modalities. This will provide a resource to recommend and educate the public about the most effective tooth whitening modality. Future studies on the adverse effects of different whitening modalities should be performed to address the effect on surface roughness, microhardness, and surface morphology. Thus, any abuse of tooth whitening that may lead to moderate to severe loss of tooth structure may be prevented.

CONCLUSIONS

DIY whitening with the use of a strawberry mixture is not an effective tooth whitening modality when compared to professionally applied in-office whitening, professionally dispensed patient-applied home whitening, and an OTC product. Both shade guides VC and BGi are related with each other and have good correlation with instrumental measurements.

Conflict of Interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Ultramorphological Assessment of Dentin-Resin Interface After Use of Simplified Adhesives

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J Tagami

Clinical Relevance

Knowing the details of ultramorphological features of the dentin-resin interface can enhance the proper selection of simplified self-etch adhesives when used in clinical practice.

SUMMARY

This study assessed dentin-resin interface integration in Class I cavities restored with simplified adhesives by using a focused ion-beam milling (FIB) and transmission electron microscope (TEM). Class I cavities (1.5-mm depth with dentin thickness of ~0.5 mm, 4-mm length, and 2-mm width) were prepared on freshly extracted, sound human molars. Two all-in-one adhesive systems (Scotchbond/Single Bond Universal [SUD] and Xeno-V$^+$ [X5D]) were used and compared with a two-step etch-and-rinse system (Prime&Bond NT [NTD]). The adhesives were applied according to the manufacturers' guidelines. A universal resin composite (Filtek Z350 XT Universal) was used to restore the cavities in one bulk filling and was irradiated at 550 mW/cm^2 for 40 seconds by a quartz-tungsten-halogen light (Optilux 501). After exposure to liquid nitrogen coolant, the specimens were milled to nanoscale thickness by FIB to view and then assess the area of dentin-resin interface by TEM. Unlike the unfilled X5D, a noticeably smooth transition zone at the dentin-resin interface was shown for the SUD and NTD adhesives. The SUD demonstrated an uneven hybrid layer with clearly demineralized collagen bundles. Ultramorphologically, dispersed needlelike apatite crystals were detected within the partially demineralized dentin or the hybrid layer of both compositionally different all-in-one simplified adhesives. Conversely, these crystals were entirely absent from the hybrid layer of the etch-and-rinse NTD adhesive. In the X5D group, a bright band was noted beneath the hybrid layer. The methacryloxydecyl dihydro-

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gen phosphate monomer containing ultramild self-etch adhesive (SUD) was still validated in terms of its capability in dentin adhesion.

INTRODUCTION

The efficiency of a direct composite restoration is affected mainly by the good sealing ability of adhesive resins.[1] Bonding to dentin is more difficult because it is a highly organic structure made of collagen and smear deposits as well as water.[2] Therefore, effective bonding of resin-based restorations to dentin is more challenging compared with that of enamel.[3] Adhesion to dentin can be attained by micromechanical hybridization, which involves infiltration of the collagen fibrils and dentinal tubules by the adhesive resin, resulting in formation of a homogeneous and strong hybrid layer.[3] The hybrid layer is a mixed area of conditioned collagenous dentin impregnated with an adhesive resin monomer.[3] The main reason that inadequate sealing develops following dental composite restoration is poor bonding between the resin and dentinal tissue, which can be evidenced by rapid marginal degradation and early debonding.[4,5] This may arise from inadequate entanglement of dentinal collagen by the resin, resulting in microgaps.[6] To maximize the dentinal hybridization process, different types of adhesive resins have been developed to allow better wettability of dentin and complete resin infiltration, thus yielding a superior adhesion.

Dental adhesive resins have been improved with fast advancement in chemical components and mode of adhesion. Simplified two-step etch-and-rinse systems were developed in the late 1990s to overcome the technique sensitivity of three-step etch-and-rinse systems.[2] The two-step etch-and-rinse bonding strategy combines hydrophilic primer and hydrophobic adhesive resin into one bottle, maintaining an initial step of a separate total etch. Etching is necessary to remove the smear layer, demineralize the dentinal tissue, and expose dentinal tubules for infiltration by the resin.[2,6] Self-etch adhesives were introduced by incorporating all three components of the adhesive (etching, priming, and bonding) in one bottle.[1] These are called all-in-one simplified adhesives, which is the most common, recent formulation for bonding agents.[6] Nowadays, they are used widely in the dental profession due to their easy, fast application; lesser risk of technique sensitivity; and acceptable clinical performance.[7] This approach to adhesives is based on the use of acidic monomers that concurrently condition and prime dental tissues without rinsing.[8] It is performed by dissolving and entangling the smear and the dentin layers as substrates for bonding according to the potency of the acidic monomer's pH.[1,9] These functional acidic monomers can dissolve the smear layer by demineralizing the superficial dentin and then incorporating adhesive resin into that mixture, which will be polymerized after exposure to light irradiation.[9] It is claimed that this approach is clinically efficient, in essence reducing the working time and technique sensitivity.[10]

Even though self-etch adhesive systems minimize technique-sensitivity during their clinical application, some of them did not absolutely enhance bonding effectiveness to dentin.[11] This could be related to an increase in their hydrophilicity or to the presence of a combined blend of hydrophilic and hydrophobic monomers that may compromise each other's function, resulting in inadequate clinical durability.[10,12] Notwithstanding a considerable improvement in adhesive resins, the bonded interface between two compositionally different substances remains the weakest area of tooth restoration. Therefore, studying the nanomorphological features of the dentin-resin interface zone by an accurate device is essential to knowing the side effects of the debonding, and it can be valuable in the innovation of dentin bonding agents.

An ultramicrotomy is commonly used to cut hard-substance samples such as dental tissues and dental composite materials for such characterization by either transmission electron microscope (TEM) or scanning electron microscope (SEM).[13,14] However, these microscopic examinations require a special set of sample-preparation procedures in order to prepare ultrathin sections. An accurate and more specific milling technique based on the ion beam has been developed for ultrathin sample preparation and structural pattern fabrication: focused ion-beam (FIB) milling.[15] This milling technique is efficient and fast and can possibly minimize some present drawbacks of the ultramicrotomy technique. It is associated with the great energy density of ions originating from a gallium source.[16] The resultant ion beam allows thinning of a homogeneous or heterogeneous structured sample into an ultrascale thickness with definite clean cutting and an artifact-free, less-distorted section.[17] Nanoscale sections of dentinal tissue samples can be prepared by FIB, and such sections can enhance imaging of structural surfaces and interfaces by a TEM to reveal the bond effectiveness of adhesive resin to dentin.[18] It is a perceptive high-quality imaging that is most com-

Table 1: *Manufacturers' Composition of Dentin Bonding Agents*

Material	Code	Type	Composition	Batch No.	Manufacturer
Filtek Z350 XT (Filtek Supreme Ultra or Filtek Supreme XTE)	Z35	Nanofill	Bis-GMA, TEGDMA, Bis-EMA, UDMA, PEGDMA, silane treated ceramic, nonagglomerated/ nonaggregated silica and zirconium dioxide nanofiller (MPS = 20 and 4-11 nm, respectively), nanocluster (MPS = 6-10 μm) and filler content of 72.5% by wt.	N234311	3M ESPE Dental Products, Seefeld, Germany
Scotchbond Universal (Single Bond Universal)	SUD	All-in-one self-etch	10-MDP, phosphate monomer, dimethacrylate resins, HEMA, methacrylate-modified polyalkenoic acid copolymer, initiators, filler, silane, initiators, ethanol, water	476114	
Xeno-V$^+$	X5D	All-in-one self-etch	Bifunctional acrylic amides, acrylamide alkylsulfonic acid, "inverse" functionalized phosphoric acid ester, acrylic acid, camphorquinone coinitiator, butylated benzenediol, water, tertiary butanol, stabilizers	1111002727	Dentsply DeTrey GmbH, Konstanz, Germany
Prime&Bond NT	NTD	Two-step etch-and-rinse	Di- and trimethacrylate resins, functionalized amorphous silica, PENTA, photoinitiators, stabilizers, cetylamine hydrofluoride, acetone	0911001257	

Abbreviations: Bis-EMA, bisphenol A diglycidyl ether dimethacrylate; Bis-GMA, bisphenol A diglycidylmethacrylate; HEMA, 2-hydroxyethylmetacrylate; MPS, mean particle size; PENTA, dipentaerythritol penta acrylate monophosphate; TEGDMA, triethylene glycol dimethacrylate; 10-MDP, 10-methacryloxydecyl dihydrogen phosphate; UDMA, urethane dimethacrylate.

monly used in investigating biological water-based tissues and nonbiological materials.[18,19]

Studying the nanomorphological feature at the dentin-resin interface after treating the dentin with bonding agents is critical to understanding the quality of their adhesion. Therefore, the objective of this study was to assess ultramorphological features of the dentin-resin interfacial area by the FIB-TEM technique following the use of two different compositionally based, simplified all-in-one self-etch adhesives and compare them with a two-step etch-and-rinse adhesive.

METHODS AND MATERIALS

Materials Used

Three dental adhesive systems (multi-mode or universal adhesive, Scotchbond/Single Bond Universal Adhesive [SUD; 3M ESPE Dental Products, Seefeld, Germany]; all-in-one self-etch, Xeno-V$^+$ [X5D; Dentsply DeTrey GmbH, Konstanz, Germany]; and two-step etch-and-rinse, Prime&Bond NT (NTD; Dentsply DeTrey]) as well as a nanofill universal composite (Filtek Z350 XT Universal, 3M ESPE) were used in this study. Table 1 describes the materials used and their composition.

Specimen Preparation

Freshly extracted, sound human third molars were used in this study after patients signed an informed consent in the oral surgery department. The extraction was done for candidates between 19 and 24 years old at one month before the launch of the study. The selected teeth were initially and thoroughly scaled of any organic debris using ultrasonic and periodontal hand scalers and then cleaned by damp-pumice slurry and a rotary bristle-brush mounted on a low-speed handpiece. The teeth were then stored in a 0.02% sodium azide–containing solution at 4°C until the time of their use to avoid bacterial growth and dehydration. Then, they were immersed in distilled water for one week at 23°C and examined for the existence of any microstructural defects or cracks that would require them to be disposed.

A conventional Class I cavity 1.5 mm deep with enough remaining dentin thickness, 4 mm long, and 2 mm wide was prepared on the occlusal surface of the tooth by a coarse flat-ended cylindrical diamond bur (837, Komet, Brasseler Lemgo, Germany) using a high-speed handpiece with copious water coolant and then finished with a fine diamond bur (8830L, Komet). The depth of the cavity into dentin was

Table 2: *Mode of Dentin Bonding Agent's Application*

Adhesive System	Mode of Adhesive's Application
Scotchbond Universal (Single Bond Universal)	1. Rub one coat of the adhesive onto the entire prepared cavity for 20 s with a disposable applicator.
	2. Subsequently dry the cavity for 5 s with a soft airstream till evaporation of solvent is indicated by no movement of the adhesive film.
	3. Irradiate with light for 10 s.
Xeno V$^+$	1. Apply one ample coat to wet the cavity with gentle agitation for 20 s.
	2. Air dry thoroughly with relatively strong air pressure for 5 s to evaporate solvent until there is no movement of the film.
	3. Light cure for 20 s.
Prime&Bond NT	1. Apply the acid etching (Conditioner 36 etching gel, Dentsply DeTrey) by a syringe needle tip for 15 s on the prepared surface.
	2. Rinse the gel and the conditioned surface with copious water spray and/or aspirator tube for 15 s and then dry slightly for 5 s, leaving a visibly moist surface.
	3. Apply one ample coat of the adhesive to wet the prepared tooth surface and leave undisturbed for 20 s.
	4. Gently dry with the mild oil and moisture-free air spray syringe for 5 s to have a uniform shiny surface and to avoid dentin dehydration and collagen collapse.
	5. Light cure for 10 s.

verified by a graded periodontal-probe. The prepared cavity was thoroughly cleaned with pumice and water and flushed gently with air to receive the restoration.

The Restorative Treatment

The prepared teeth were randomly distributed according to the brand of adhesive bonding agent used. The application of these adhesive bonding agents according to their respective manufacturer's instructions are displayed in Table 2. The light irradiation of the adhesive layer was carried out by a visible quartz-tungsten-halogen light (Optilux 501, Demetron/Kerr Corp., Danbury, USA) at 550 mW/cm^2 as verified with an external radiometer (Demetron/Kerr Corp., Danbury, USA).

All prepared occlusal cavities treated with the respective adhesive system were restored with a nanocomposite (Filtek Z350 XT Universal [Filtek Supreme Ultra or Filtek Supreme XTE], 3M ESPE) in one bulk filling and then irradiated by the Optilux 501 light (Demetron/Kerr) at 550 mW/cm^2 for 40 seconds. Subsequently, the polymerized resin composite was finished with a diamond finishing bur (8368.016, Komet) and polished with Astropol points (Ivoclar Vivadent, Schaan, Liechtenstein) mounted on a low-speed handpiece at 12,000 rpm under copious water coolant. Thereafter, the restored samples were stored in a humid state at room temperature for one week preceding the milling process.

FIB Milling and TEM Evaluation

The roots of the prepared teeth were cut away and the specimens were trimmed from the buccal side longitudinally parallel to the opposing wall to expose the dentin-resin interface. The trimming was performed manually on 600-grit silicon carbide papers under water coolant and then sequentially finished with a series of these abrasive papers (800-, 1000-, 1500-, and 2000-grit). The specimens were cleaned in an ultrasonic water bath for three minutes between each abrasive paper grit and then polished in a descending series with diamond pastes (6, 3, 1, 0.25 μm) under running water. After polishing, the interfacial area of the specimen was selected by direct observation with a confocal laser scanning microscope (1LM21H/W, Lasertec Co, Yokohama, Japan) and then imaged at 100× magnification.

Each sample was sputter-coated with a 500-nm layer of tungsten in order to permit enhanced exposure of the resin-dentin interface to the FIB milling procedure (FB2200, Hitachi, Tokyo, Japan) and thereafter for microscopic and morphologic feature patterns evaluation. The FIB milling procedure was conducted in a cryogenic state (Cryo-FIB) using a liquid nitrogen coolant reservoir attached to a specimen milling stage. FIB milling is based on the impact interaction between profound ions produced mainly by a gallium source that is accelerated in a high electrical voltage field of 10 kV, and the tooth surface's sample yielding a fine beam. The initial milling current by the FIB for bulk slicing was obtained at 4 nA, yielding a 3-μm thick specimen. On the other hand, the current beam applied to attain fine milling of a specimen slice to a nanoscale thickness of 100 nm was 10 pA at 10 kV. This high-energy beam can precisely mill and cleanly cut both the tooth-tissue surface and the resin filler of the composite into nanofoils. Moreover, this current is needed to view this ultrathin slice and to characterize the sectioned area at the dentin-resin interface by subsequent TEM for better assessment.

The ultrathin FIB-milled specimens (100 nm thick) were examined by the TEM (TEM HT7700, Hitachi) operating at 80 kV. The TEM samples were produced *in situ*, which involved a micromanipulator to transfer the thinned section to a TEM grid inside the vacuum chamber.[17] The resin-dentin zone of interest was identified at a range of magnification between 10,000× to 80,000×.

RESULTS

The FIB cross-sectioning technique clearly disclosed typical representative micrographs that were obtained from the X5D, SUD, and NTD nondemineralized and nonstained sample groups (Figures 1, 2, and 3, respectively). Unlike conventional microtomes, all FIB-sliced specimens had a limited range of view and uniform sample thickness (~100 nm). Moreover, preparing the specimens in a cryogenic state had preserved the morphology of the interface with no signs of beam damage.

Generally, the obtained sections showed a homogeneous adhesive layer over the treated dentin surface. Excluding the unfilled resin in X5D (Figure 1), the interface between the filler particles and resin matrix of the other adhesives was markedly smooth (Figures 2 and 3). We found it interesting that although the nanofillers within the adhesive layer were clearly perceived in SUD and NTD, these clusters were not perceived to be infiltrating the interfibrillar spaces of the hybrid layer. Furthermore, similar ultramorphologies were observed among the self-etch adhesive groups. The transitional zone was different in each self-etch group, and it was filled with dispersed needlelike apatite (Ap) crystals within the partially demineralized dentin or the hybrid layer (200-800 nm thick) (Figures 1d and 2d,f). However, these Ap crystals were completely removed from the hybrid layer of the etch-and-rinse adhesive NTD group (Figure 3d).

In the X5D group, the adhesive layer did not show any sign of fillers; however, it was associated with variable round voids within the adhesive layer (Figure 1a,b). The actual hybrid layer and the resin-infiltrated smear layer were difficult to distinguish from each other (Figure 1b-d). The ultramorphological examination of this layer demonstrated a mixture of remnants of smear layer and traces of demineralized Ap crystals that had lost their needlelike characteristics (Figure 1e). Moreover, this layer had an uneven thickness ranging from 500 to 800 nm with consistent underlying dentin surface to some extent. A submicron hiatus (clear bright band) was found between the hybrid layer and dentin surface (Figure 1d,e). The underlying resin-infiltrated dentin layer presented a complex of microstructure intermingled with Ap crystals, micropores, and nested demineralized, banded collagen bundles with gap and overlap zones (Figure 1f). It is important to mention there was no sign of artifact due to beam damage related to preparation with a high-energy ion beam.

For the SUD group, there appeared to be a uniform distribution of the inorganic nanofillers within the light-cured adhesive resin matrix and a homogeneous interfacial adhesion between the dentinal surface and the bonding resin layer (Figure 2a). Furthermore, there was a smooth transition from the adhesive layer to the hybrid layer without separation or microporosities. However, the hybrid layer was uneven and showed a small remnant of the smear layer, and the treated dentin surface was markedly smooth (Figure 2b,c). At higher magnification, an abundance of crisscross needlelike Ap crystals can be clearly observed above the treated dentin surface and within the hybrid layer, which ranged from 200 to 500 nm in thickness (Figure 2c,d,f). Moreover, banded demineralized collagen fibrils with gap and overlap zones were clearly observed along with Ap crystals and other dental microstructures in the dentin complex (Figure 2c-e). However, this group did not show any hiatus space beneath the hybrid layer as was noted in Figure 1.

In the NTD group, it was clearly detected that the nonaggregated nanofiller-sized particles were highly and homogeneously distributed within the adhesive resin matrix (Figure 3). It was evident that the phosphoric acid treatment had completely removed the smear layer, resulting in a grossly etched and dented underlying dentin surface in the form of nano-irregularities or “shredded” appearance of the dentin. This can produce a layer of mineral-depleted collagen fibrils that are infiltrated with resin monomer (Figure 3a-e). Although the obtained sections revealed an intimate interaction with a relatively sharp transitional zone between dentin-resin substrates, the interaction zone was filled with

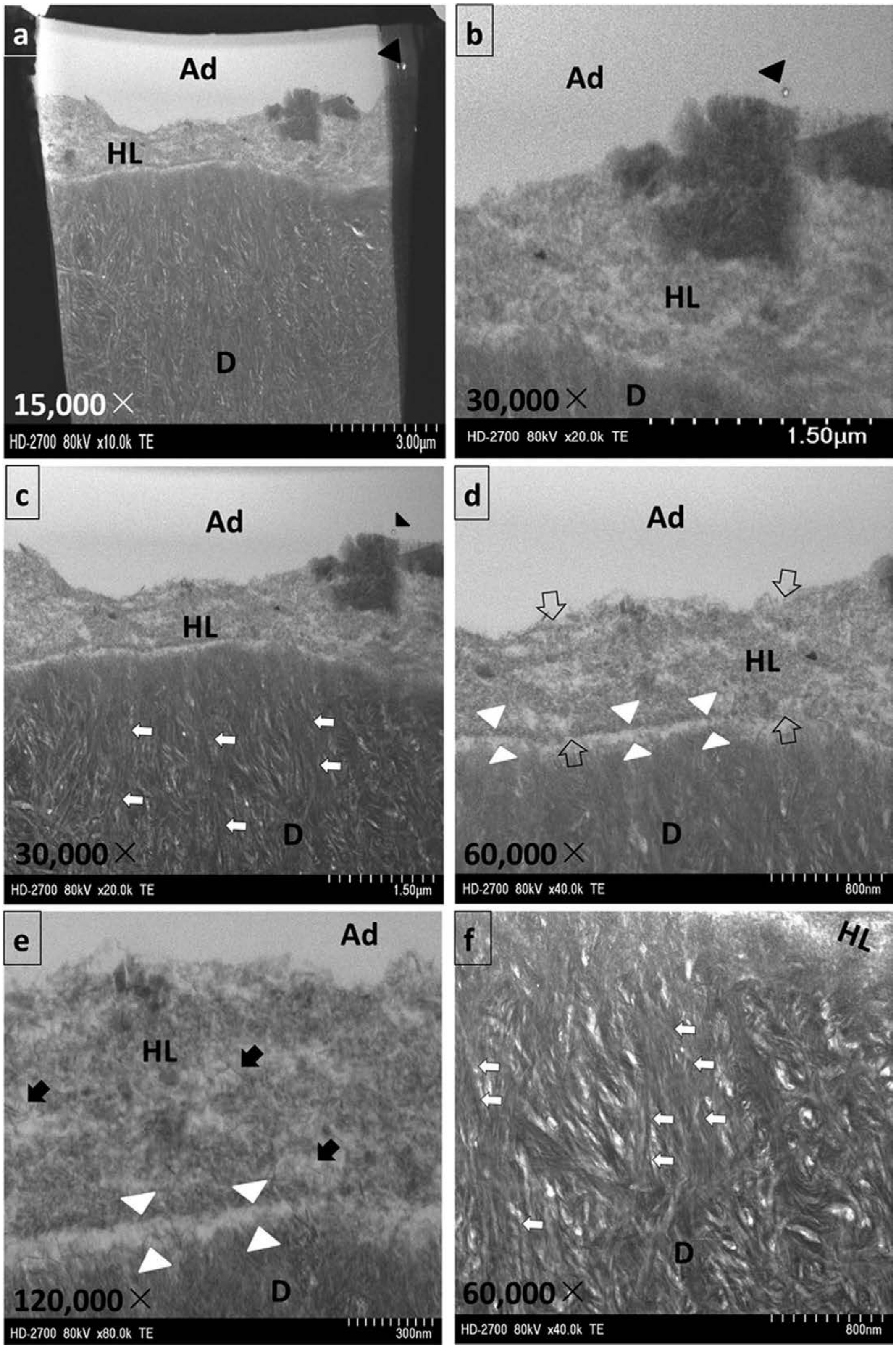

Figure 1. *Representative unstained TEM micrographs of the X5D group. (a): Low magnification of the sectioned adhesive-resin interface. The black triangles are pointing to representative round voids of phase-separation phenomena within the adhesive. (b): The round void indicated by the black triangle is representing phase separation within the adhesive. (c): The adhesive resin layer was clear (electron-lucent) and lacked inorganic filler. The underlying dentin complex showed the contrast of collagen and polymer-based phases (white arrows). The black triangle is showing the phase-*

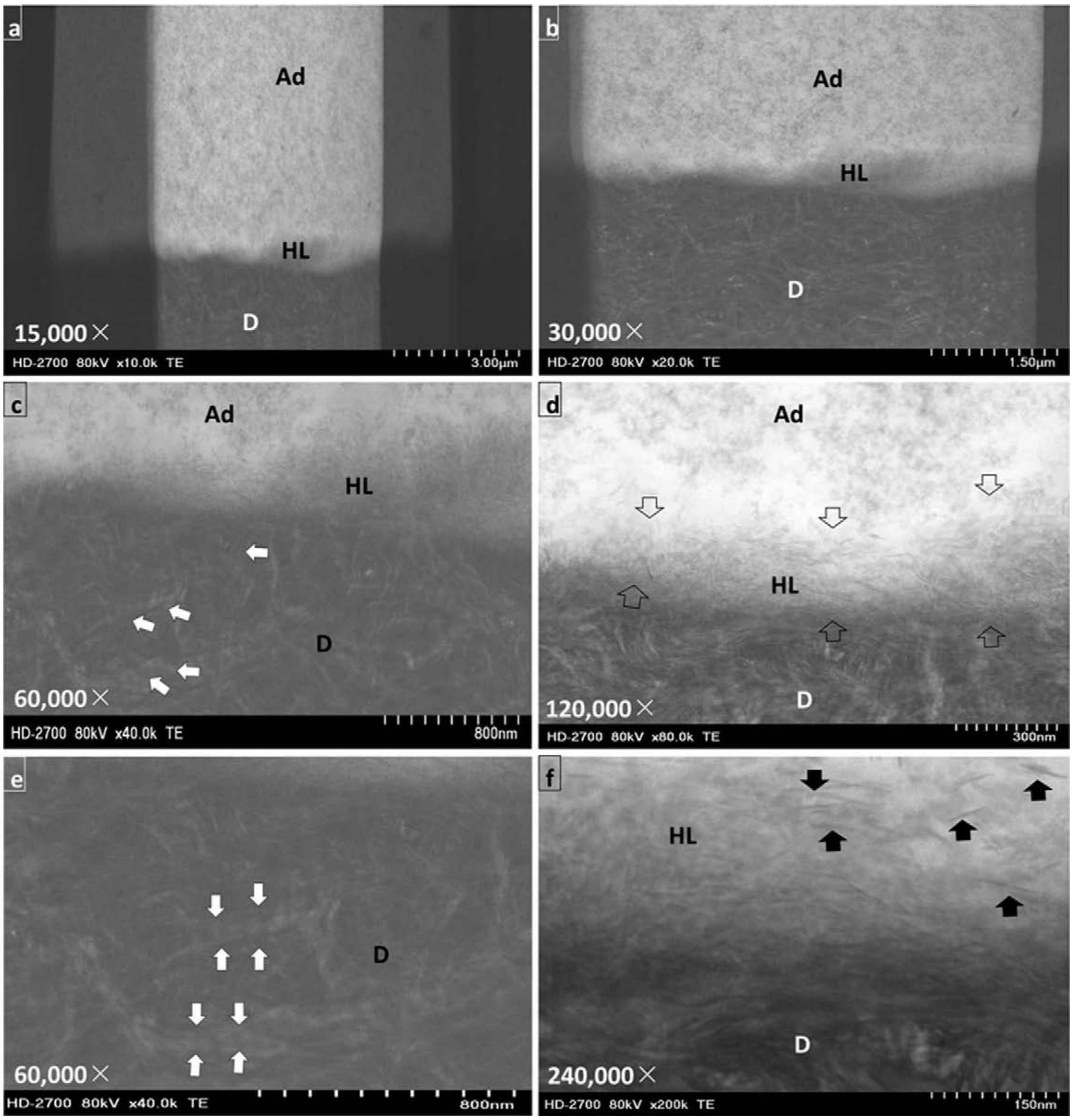

Figure 2. *Typical representative TEM micrographs of the SUD group. (a): Low magnification image showed the resin infiltrating dentin with hybrid layer formation. (b): TEM observation revealed the adhesive layer with homogeneous scattered nanofillers within the adhesive matrix. (c): A gradient transitional zone was detected from the adhesive resin to the dentin surface, ranging from 200 to 500 nm. The dentin organic phase with demineralized collagen bundles (white arrows) were clearly detected superficially and were not affected during FIB preparation. (d): At higher magnification, crisscross needlelike hydroxyapatite crystals within the hybrid layer with some remnants of the smear layer were clearly disclosed between the pointed hollow arrows. (e): The demineralized collagen bundles are indicated between the white arrows. (f): At high magnification of the hybrid layer, the black arrows are pointing to the needlelike hydroxyapatite crystals. Abbreviations: Ad, Adhesive resin; HL, hybrid layer; D, dentin.*

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separation phenomena. (d): The transitional zone from the adhesive layer to the dentin complex through the hybrid layer (500~800 nm) with undefined contents was clearly distinguished between the illustrated hollow arrows. A bright band of interfacial space presented between the white triangles. (e): Higher magnification of the hybrid layer revealed some scattered Ap crystals (black arrows) that had lost their needlelike shape with the peculiar appearance of the remnants of the smear layer. The bright band beneath the hybrid layer (white triangles) could be attributed to the water movement into the space. (f): The microstructures of the dentin complex presented micropores, scattered Ap crystals, and nested resin-infiltrated collagen bundles. Preparing the specimens in a cryogenic state had preserved the resin-infiltrated dentin organic phase. The effect of moderate self-etch adhesive in demineralizing the mineralized collagen bundles with a gap and overlap zones (white arrows) was clearly perceived at a deeper level. Abbreviations: Ad, adhesive resin; HL, hybrid layer; and D, dentin.

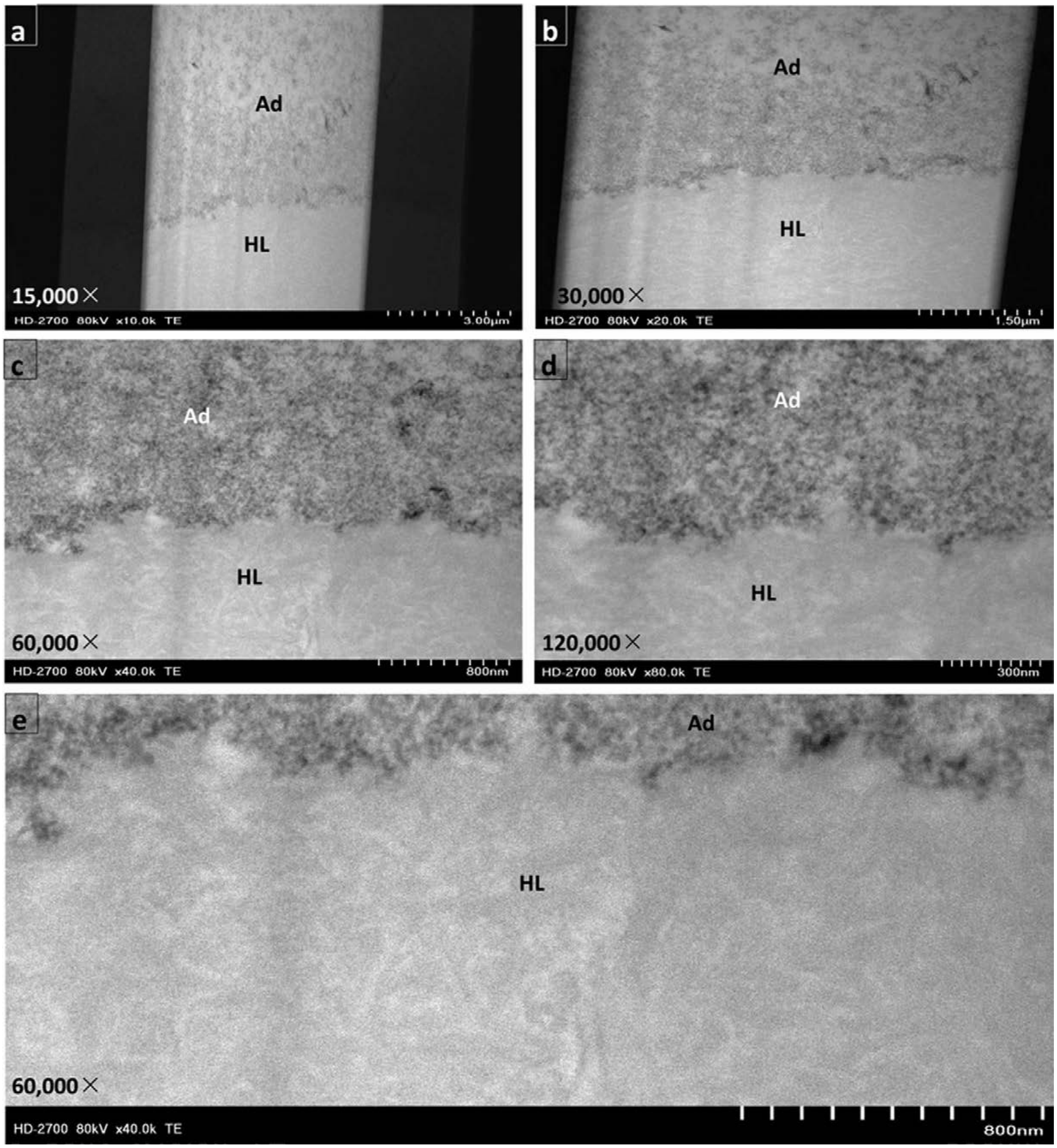

Figure 3. *Unstained TEM micrographs of the interfacial area of the NTD group. (a): The low magnification of the obtained microsample showed adhesive infiltrated dentin complex with thick hybrid layer formation (>3μm). (b): The unstained images revealed homogeneously scattered nonaggregated nanofillers throughout the adhesive layer intermixed with remnants of demineralized dentin microstructures and without penetration of the underlying hybrid layer. The sharp transitional zone from adhesive layer to dentin complex was markedly perceived. (c): The underlying hybridized dentin was mineral depleted with an electron-lucent appearance, and no remnants of the smear layer were distinguished. (d) Phosphoric acid conditioning had washed out the apatite crystals and produced nano-irregularities or shredded dentin surface with indistinguishable appearance of the underlying denuded dentin collagen. (e): The unstained images revealed homogeneously scattered nonaggregated nanofillers throughout the adhesive layer mixed with remnants of demineralized dentin microstructures and without penetration of the underlying mineral-depleted hybridized dentin layer. Phosphoric acid conditioning had washed out the Ap crystals and produced nano-irregularities or a shredded dentin surface with indistinguishable appearance of the underlying denuded dentin collagen. Abbreviations: Ad, adhesive resin; HL, hybrid layer.*

fillers and undefined remnants of demineralized dentinal microstructure, and it was very difficult to define or detect the demineralized Ap crystals (Figure 3b,c). It is worth mentioning that the hybrid layer was electron-lucent and thicker than that in other groups, with an appearance of radical demineralization and undefined dentin collagen complex (Figure 3d,e).

DISCUSSION

Ultramicrotomy has been routinely applied as a conventional technique for preparation of TEM samples. More recently, cryogenic ultramicrotomy was introduced to limit the need for sample fixation. However, both preparation techniques are unable to maintain true structural features, which hampers their broad applications. Moreover, in these techniques, the cellular structures are generally compressed in the direction of cutting. Some investigators have overcome this distortion by applying the FIB preparation method.[20] Other hindrances to ultramicrotomy preparation relate to nonuniform slice thickness and the inability to choose specific areas of interest.[21,22]

FIB is a site-specific milling tool that had been used in monitoring and manipulating the structure of tissues and materials on a nanoscale.[21,22] The main advantages of this technique are the accuracy in site selection and the cutting and thinning directions for the sample that enable precise investigation when FIB is combined with TEM. Formerly, FIB had been implemented using a conventional cross-sectioning technique, which later was modified and simplified with the introduction of the *in situ* microsampling method.[17,23,24] For dental application, it has been reported in the literature that use of the latter method resulted in a much higher sample yield during the preparation of ivory dentin for ultramorphological examination.[19,21] Moreover, combining the FIB modality with TEM facilitated the investigation of the hard materials (ie, composite fillers, as well as brittle phases; enamel and dentin) and the tooth-resin interaction for clinically relevant cavity setups, in contrast to conventional ultramicrotomy.[21]

During cavity preparation, a coarse diamond bur was used for cavity outlining and extension followed by finishing with a fine diamond bur. Apart from the aggressiveness of the diamond burs, the reported studies in the literature showed no difference in the created smear layer between carbide bur and fine-diamond bur, with comparable bonding strength.[24] It is noteworthy that the same resin composite was used to restore the cavities for the purpose of standardizing the experiment.[25]

Bonding to dentin is more challenging than bonding to enamel because it requires great attention. This is mostly attributed to the complex hydrated structure of dentin[26,27] and to the water that can diffuse from hydrated dentin through water pathways in the hybrid layer to reach the adhesive-composite interface.[28] The characteristics of the dentin smear layer are dependent upon the surface-conditioning treatment. Moreover, preparation of the dentin cavities using diamond burs resulted in undulated surfaces that promoted debris stagnation.[29,30] Additionally, the pH values of the conditioning agents have a great influence on the remaining smear layer covering the dentinal surfaces.[29,30] Therefore, the pH values of the self-etch adhesive and the method of dentin cutting might possibly contribute to the detected variation in the smear layer thickness as reported in the literature.[29,30] Moreover, different adhesive formulae were selected with different monomers and solvents to characterize the hybridized dentin-adhesive interaction zone.

The clustered nanofillers within the adhesive layer that neither infiltrated the hybrid layer in SUD nor the hybridized dentin complex in NTD group were clearly perceived (Figures 2 and 3). These findings correspond with a previously reported study in the literature that the formed filler clusters are too bulky to infiltrate the spaces of the hybrid layer.[31] Furthermore, retention of the ground substance within the demineralized intertubular collagen matrix can hamper the infiltration of the nanofillers.[31] However, these nanofillers may contribute to the higher viscosity of the filled adhesive resin. This in turn will produce a sufficient adhesive thickness over the hybrid layer that may stabilize it.[32-34]

For the two all-in-one adhesive systems studied (X5D and SUD), FIB-TEM images revealed a variable transitional zone between the adhesive resin and the underlying treated dentin representing their respective hybrid layers. Moreover, the detected micropores in the hybridized dentin were similar to those with comparable dimensions and dispersion between Ap crystals in the dentin sections that were found by ultramicrotomy-TEM.[22,35] The vivid appearance of the organic components of the dentin could be attributed to the minimized beam-damaging effect achieved by conducting FIB in a cryogenic state, which enabled visualization of the resin-infiltrated demineralized collagen with the characteristic gap and overlap zones (Figures 1 and 2).[21,36] However, strong phosphoric-acid etching in the NTD group completely or predominantly deprived dentin collagen of Ap crystals, leaving the organic component exposed for the infiltrating adhesive resin (Figure 3).

X5D is a 2-hydroxyethyl methacrylate (HEMA)-free all-in-one self-etch adhesive that has an improved chemical formula from its predecessors and is optimized without acrylic acid inclusion. The absence of HEMA in this category may reduce water uptake and thus hydrolysis at the adhesive interface.[37,38] However, it may induce phase separation between hydrophilic and hydrophobic components, given that it was spotted at some areas within the adhesives above the hybrid layer (Figure 1a,b). According to the manufacturer, the adhesive matrix lacks nanofillers, which explains the clear appearance of the adhesive layer and relative reduced viscosity. Moreover, the increased acidity of the functional monomers (ie, functionalized phosphoric acid ester and acrylamide-alkylsulfonic acid) that reduced the pH value (pH = 1.3) can explain the undistinguished content of the hybrid layer, which had the vague appearance of partially demineralized Ap crystals in shallow and deep dentin collagen (Figure 1b-f). Another point to be mentioned is that most of the reported TEM studies on HEMA-free adhesives displayed variable degrees of submicron hiatus space beneath and within the hybrid layer, which agreed with the results obtained in the X5D group (Figure 1d,e).[29,39,40] This space could be ascribed to the water movement from the underlying hydrated dentin complex toward the adhesive resin[28] and/or to the incomplete removal of the solvent (tertiary butanol), which requires relatively more pressure (vapor pressure = 4.1 kPa at 20°C) to evaporate than the solvent in SUD (ethanol; vapor pressure = 5.8 kPa at 20°C) and NTD (acetone; vapor pressure = 24 kPa at 20°C) during the drying phase.[41]

The prepared cavities in the SUD group were treated using one-step self-etch adhesive (Scotchbond Universal adhesive) that had shown a gradient transitional zone from the adhesive resin to the dentinal surface through the hybrid layer, with some nanofillers dispersed within the adhesive matrix. Unlike in X5D, this adhesive formula contains HEMA that may inhibit phase separation by homogenizing the hydrophilic and hydrophobic phases.[38] Also, the high pH used in SUD (pH = 2.7) comprised of methacryloxydecyl dihydrogen phosphate (MDP) functional monomer, which has a high affinity to bond ionically to partially demineralized Ap crystals, can preserve their needlelike appearance without complete removal of the smear layer at the interdiffusion zone.[42] Although it was not the aim of this study, the author speculates this MDP-apatite interaction would result in formation of MDP-calcium salts in the form of nano-layering assemblies at higher magnifications, as was reported by other studies.[43,44] A point of interest in this category is the ability to maintain the uniformity of the dentin surface with limited demineralization depth and a shallow resin-infiltrated collagen demineralization process, which is attributed to the mild acidity of this adhesive system and may account for dentin bonding stability.[45]

In NTD, the obtained TEM sections confirmed the claims of the high dispersion of the nonaggregated nanofillers within the adhesive resin matrix that were clearly perceived in the interaction zone mixed with remnants of demineralized dentin microstructures. This adhesive is an acetone-based two-step etch-and-rinse adhesive system and uses strong phosphoric acid as a surface conditioning, before adhesive application, that can completely eliminate the smear layer as well as demineralize the underlying dental tissues, leaving denuded collagen behind. It contains acidic phosphonated monomer (dipentaerythritol penta acrylate monophosphate) that has the ability to interact with calcium ions left on the dentin surface after collagen demineralization.[46] The inclusion of acetone in its chemical formula will ensure adhesive infiltration by displacing water remnants from the dentin surface and act as a "water-chaser."[33] However, this volatile solvent has a relative high vapor pressure, which makes this system more technique sensitive during the restorative procedure. The effect of phosphoric acid conditioning was apparent in the form of nano-irregularities or shredded dentin surface with pronounced hybridized resin–infiltrated dentin complex that appeared electron-lucent in the TEM sections, indicating the absence of a crystalline phase within these regions. These findings coincide with other reports in the literature.[42,47] Taking into consideration the adhesive viscosity and deep dentin demineralization in etch-and-rinse systems in general, the enzyme activation of proteolytic matrix metalloproteinases is expected to be induced and long-term bond durability would be affected.[48] However, this is unlikely to be seen in self-etch adhesives that have the ability to etch and penetrate simultaneously, which would result in resin-collagen fixation and consequent long-term bond stability.[45,48,49]

Within the limitations of this study, including time consumption, reduced field of view, and technique sensitivity that requires skilled personnel to operate the FIB system, the ultrastructural characterization of the changes in adhesive resins over the long-term should be considered in future FIB-TEM studies.

CONCLUSIONS

It can be concluded that FIB preparation methodology is a unique technique for preparation of hard materials and brittle phases and can substitute for conventional methods. Although this technique is very sensitive and may be prone to some artifacts, it showed a lot of potential for future research when performed in a cryogenic state. Interfacial assessments showed the etching pattern differences of the self-etch adhesives in comparison with an etch-and-rinse adhesive system and their marked influence on the organic phase of dentin. Incorporating MDP as a functional monomer to the newly introduced self-etch adhesive confirmed its competence as a dentin bonding mechanism. The overall results of this study provided a valuable insight that should be considered during adhesive selection in routine dental practice.

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Conflict of Interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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